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THE INFLUENCE OF DRYING ON SOIL BUFFERING IN RELATION TO AGGREGATION AND OTHER FACTORS[1]

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INTRODUCTION

Acidity, buffering, base exchange, and exchange capacity are obviously all closely interrelated, and are recognized as some of the most important fundamental properties of soils. A great deal of work has been done to obtain a better understanding of these properties and much progress has been made. Since the work of Bradfield (*5*) [2] it has gradually become recognized that complex aluminosilicate minerals, or clay minerals, are one of the main sources of soil acidity and buffering as well as of physical activity in soils. He and others (*3, 20*) have shown that much of the behavior of these purified clay minerals is like that of weak acids. The other major source of reserve acidity and buffering of soils is recognized as organic in origin (*3, 4, 21*). The buffering powers of organic compounds are often so great that a small percentage of organic matter dominates the entire exchange and buffering properties of many soils (*21, 32*).

If the complex organic materials were omitted it would seem that comparatively simple expressions might well be worked out for acidity, buffering, and exchange capacity. But after a number of years of work by many investigators there is still no widespread agreement as to the fundamental definition of exchange capacity and no absolute method for making buffer measurements that are directly applicable in the field.

As a practical procedure for determining a standard relative exchange capacity value the neutral ammonium acetate method is probably the most widely used and the most useful (*31*). However, it is recognized that exchange capacity values obtained by this or by other standard laboratory methods cannot be used as an absolute measure of the lime or base content at pH 7 in the field (*20, 22, 24, 25, 28*). Moreover, Bradfield (*6*) has shown that soils and clays have a strong buffering capacity above pH 7, and he has suggested that a more satisfactory, practical end point for exchange capacity is the equilibrium point with calcium carbonate in an atmosphere with a CO_2

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[2] Italic numbers in parentheses refer to Literature Cited, p. 282.

concentration of the air. This end point would be at a pH value of about 8.2.

A different end point has been used by Baver (*3*) in carrying through the analogy between clays and weak acids. His use of the flex points of soil-base buffer curves gives end points at variable pH values. With pure clay systems the flex points are often clearly evident, but with soils these flex points may be indistinct. Further, the simple weak acid concept does not explain the strong buffering above the flex point which is typical of many soils. Another complication in this explanation as well as in other approaches to soil buffering is the difference associated with the neutralizing ion. There is a wide difference between such buffer curves with sodium and lithium on the one hand and calcium and barium on the other, so that buffering can be expressed only by specifying the ion involved.

In practical soil liming studies it has been shown that factors of from 1.5 to as high as 3.0 must be used to convert laboratory buffer values into field lime needs, even when mixing is complete and all the lime is fine and readily available (*25*, *28*). Kappen (*14*) has noted a similar discrepancy between laboratory buffering and field behavior, but has attempted to dismiss it as probably resulting from incomplete contact in the field.

The literature on base exchange contains many indications of some sort of build-up of exchange capacity (*8*). Pierre and Scarseth (*24*) considered that $Ba(OH)_2$ treatment probably built additional exchange, and they rejected the preliminary treatment with $Ba(OH)_2$ for base exchange capacity determinations as recommended by Kelley (*15*) on this basis. They found that for limed soils the results were essentially the same with $Ba(OH)_2$ pretreatment as with the direct BaAc-NH_4Cl method, which they interpreted as indicating that liming, like treating a soil with an alkaline solution, results in a "build-up" of additional exchange complex. Kelley (*15*) considered that the $Ba(OH)_2$ treatment merely resulted in displacement of all of the potentially exchangeable H+ and he did not consider this to constitute a build-up of exchange. There are other references to the difficulty of replacing the H+ from exchange material. Kappen (*14*) discusses this at some length, and Magistad (*18*) has emphasized the extreme slowness of replacement of H+ from exchange material compared to the replacement of metallic ions, in conjunction with his argument that alkaline treatments build more exchange instead of merely releasing more H+ as indicated by Kappen.

Field data of Robinson (*26*) show that for a particular soil type there may be a significant positive relation between the pH and the exchange capacity, suggesting some influence of bases upon the exchange capacity. For samples of Hagerstown silty clay loam having little variation in organic matter content his data show a rather close relation between pH and exchange capacity by the neutral ammonium acetate method. With Dekalb silt loam the pH-base exchange capacity relation is also distinct, but in this case organic matter variations may account for a part of the correlation.

Experimental plot results reported by Salter and Schollenberger (*29*) suggest that liming may have caused an increase in the exchange capacity on 32-year-old plots. Without manure, unlimed plots show an exchange capacity of 8.01 m. e. as compared to 9.87 m. e. for limed

plots; and with manure the values are for unlimed, 8.89 and for limed, 10.51 m. e.

Drying has been considered by a number of investigators (*1*, *9*, *10*, *11*, *12*, *13*, *17*, *27*, *28*) as one of the factors that influence acidity. Most reports have indicated some decrease in pH due to drying, but some have shown an increase with certain soils. Coles and Morison (*10*) have studied the effect of drying upon acidity in considerable detail and their results are in general agreement with others reporting pH decreases. They found a considerable lowering of the pH of all mineral soils by drying at 98° C., the greatest effect occurring on the first day, but some additional lowering being evident for several days. Peat showed a slower change downward which extended out over a longer period. Coles and Morison concluded that these pH changes were reversible for mineral soils, nonreversible for organic materials. This conclusion is not entirely substantiated by their data, for the mineral soils generally failed to reach the original pH value even after a period of 57 days of soaking. In fact, there was no clear trend toward a return after about 2 weeks. But, at least, the pH change was to a considerable extent reversible with all mineral soils. Their results also showed the elimination of the drying effect by preleaching with HCl and the return on treatment with a base. This seemed to indicate that the effect of drying was due to some reaction of the exchangeable bases. However, the pH values obtained by leaching with HCl and washing with water were well below 3.0 in most cases, which might lead to some question as to whether all the excess HCl had been removed. If not all removed, then the heating might be expected to drive off some HCl and thus prevent a lowering or even cause an increase in pH. Actually, a slight increase was obtained in some cases, particularly with two samples treated with H_2O_2 as well as with HCl. The increase was more than 0.2, starting with values below pH 2.6. Exchangeable bases were decreased by drying; water-soluble Ca and P were increased.

The object of the present paper is to report data obtained from drying soils before determining the pH, especially in connection with measurements of buffering capacity; and to attempt to show how the influence of drying may help to clarify some of the indicated interrelations of pH, buffering, base exchange, and exchange capacity.

METHODS

All pH values were obtained with a glass electrode pH meter. A soil-water ratio of 1 : 2½ was used in some cases; other values were obtained on soil pastes, as indicated in the tables. Drying was accomplished in an oven at 110° C. unless otherwise indicated. Total exchangeable bases and exchange capacity were determined by the neutral, normal ammonium acetate method (*31*). Organic matter values are by dichromate oxidation (*7*).

Certain details of technique will be explained under specific headings in the results.

SAMPLES

A variety of representative West Virginia soils and subsoils have been studied. These include normal surface soils and subsoils of the gray-brown podzolic group. Clay subsoils represented are of acid

shale, limestone, and alluvial origin. Silty and fine sandy soils are derived from acid shales and fine sandstones. A sample designated as "muck" was obtained under "half-bog" conditions in a high valley draining acid shale and sandstone upland.

Most of the soils in the study are known from petrographic observation to be rich in hydrous micas. Clays of the 2:1 type are probably dominant in most samples. Base exchange studies show that the 0.002-mm. clay fraction ordinarily has an exchange capacity of about 30 m. e. per 100 gm. by the ammonium acetate method (*32*). One electrodialyzed colloidal clay, from Hagerstown subsoil, has been included in the study. Its exchange capacity is about 60 m. e. per 100 gm. One sample of commercial kaolinite and one of Wyoming bentonite are also included.

RESULTS

INFLUENCE OF DRYING ON THE PH OF VARIOUS SOILS AND CLAYS

Under West Virginia conditions the highest soil pH values are invariably obtained on moist samples. In a few cases there is no significant difference due to drying, but in most cases drying causes a marked decrease. Typical values are shown in table 1. A variety

TABLE 1.—*The influence of drying upon the pH of different soil and clay samples*

Soil No.	Description	Organic matter	pH of samples after soaking 3 hours—starting with condition indicated		
			Field-moist	Air-dry	Oven-dry
1	Gilpin silt loam (gray-brown silt loam, upland surface soil)	*Percent* 3.4	4.9	4.75	4.2
2	Dekalb silt loam	2.9	5.7	5.3	4.9
3	Gilpin silt loam	4.3		5.1	4.4
4	Holston sandy loam terrace	1.3	4.8	4.4	4.15
5	Monongahela silt loam	3.5	5.75	5.3	4.9
6 (a)	Holston loam subsoil	.2	5.1	4.85	4.5
6 (b)	Leached with HCl, washed with H_2O			3.65	3.25
7 (a)	Zoar clay subsoil	.3	5.3	4.95	4.0
7 (b)	Leached with HCl, washed with H_2O			3.7	3.4
8	Blago silt loam	12.4	4.9	4.5	3.9
9	Sandy podzol B horizon	3.3		4.4	4.0
10	Silty subsoil	1.4		4.7	3.8
11	Woodland mull	11.8		5.4	4.7
12	Electrodialyzed collodial clay			3.6	3.2
13	Commercial kaolinite			6.8	6.0
14	Sand and bentonite mixture leached with HCl and washed with H_2O			4.6	3.8
15	Same, but leached separately			3.7	3.2

of soils and subsoils are represented as well as soils leached free of all bases, a sample of commercial kaolinite, one of bentonite, and one of electrodialyzed H+clay.

INFLUENCE OF DRYING ON SOIL BUFFERING

When increments of base are added in solution to soil suspensions and allowed to stand with occasional shaking, pH results as shown in table 2 are obtained. The results are seldom completely stable with time, but after about 2 days a fairly satisfactory equilibrium is reached with most soils. A similar equilibrium time has been used by others (*19*, *20*, *25*, *30*).

TABLE 2.—*Influence of time on the pH of soil suspensions in contact with a base*

Soil	M. E. of base	pH of soil suspension after—					
		1 day	2 days	3 days	5 days	7 days	17 days
1	3.6 Ba	5.55	5.6	.....	5.45	5.45	.....
3	None	5.1	5.1	5.0	.....	.....	.....
	3.6 Ba	6.3	6.0	6.2	.....	.....	.....
	5.2 Ba	7.3	6.7	6.7	.....	.....	.....
5	3.6 Ba	6.6	6.45	.....	6.45	6.5	.....
6	3.6 Ba	8.1	7.5	.....	7.6	7.1	.....
7	None	.....	4.9	.....	.....	4.8	4.8
	1.9 Na	.....	6.0	.....	5.65	5.65	.....
	3.6 Ba	5.7	5.55	.....	5.50	5.55	.....
	3.8 Na	.....	6.6	.....	6.0	6.15	.....

It the samples are then dried at 105° C. and redispersed in water the pH values are all lower than they were before drying. Table 3 shows some typical values. This table and figures 1 and 2 show that there is usually a greater decrease in pH after additions of base than before.

TABLE 3.—*The effect of drying upon soil pH following the addition of various increments of base*

Soil	M. E. and kind of base added	pH of samples after soaking 2 days in contact with base	Immediate pH of samples redispersed in water after oven drying following the 2-day contact with base	Difference
1	0	4.75	4.2	0.55
	2.0 Ba	5.25	4.5	.75
	3.7 Ba	5.6	4.8	.8
	5.6 Ba	6.9	5.1	1.8
2	0	5.3	4.9	.4
	2.0 Ba	6.4	5.4	1.0
	3.7 Ba	6.9	5.9	1.0
	5.6 Ba	7.05	6.6	.55
4	0	4.75	4.25	.5
	2.0 Ba	5.75	4.70	1.05
5	0	5.3	4.9	.4
	2.0 Ba	6.1	5.5	.6
	3.7 Ba	6.65	5.9	.75
	5.6 Ba	7.05	6.0	1.05
6 (a)	0	5.0	4.4	.6
	2.7 Ba	6.2	5.05	1.15
	5.2 Ba	6.75	6.0	.75
	7.8 Ba	7.3	6.75	.55
	0	5.05	4.4	.65
	2.4 Na	6.4	5.4	1.0
	4.9 Na	7.05	6.0	1.05
	7.4 Na	7.7	6.6	1.1
6 (b)	HCl leached and washed with H_2O	3.65	3.25	.40
7 (a)	0	5.0	4.0	1.0
	1.8 Ba	5.4	4.3	1.1
	3.6 Ba	5.75	4.8	.95
	7.2 Ba	7.2	5.95	1.25
	12.7 Ba	8.3	7.2	1.1
	0	4.95	4.0	.95
	2.8 Na	6.0	4.4	1.6
	3.9 Na	6.65	5.05	1.6
	4.9 Na	6.9	5.25	1.65
	5.8 Na	7.3	5.5	1.8
	7.8 Na	7.95	5.95	2.0
	9.7 Na	8.5	6.7	1.8
	11.7 Na	8.95	7.2	1.75
7 (b)	Leached free of base with HCl and washed with H_2O	3.7	3.4	.3
8	0	4.5	3.9	.60
	3.6 Ba	5.05	4.05	1.0
10	0	4.7	3.8	.9
	4.2 Ba	6.7	5.5	1.2
	6.9 Ba	7.6	6.95	.65
	7.0 Ca	7.6	7.0	.6

TABLE 3.—*The effect of drying upon soil pH following the addition of various increments of base*—Continued

Soil	M. E. and kind of base added	pH of samples after soaking 2 days in contact with base	Immediate pH of samples redispersed in water after oven drying following the 2-day contact with base	Difference
11	0	5.4	4.7	.7
	4.7 Ba	5.65	4.8	.85
	19.8 Ba	6.3	5.1	1.2
	25.0 Ba	7.0	5.4	1.8
12	0	3.55	3.35	.2
	8.2 Ca	4.05	3.45	.6
	16.4 Ca	4.35	3.5	.85
	24.4 Ca	4.65	3.75	.9
	33.0 Ca	4.9	4.2	.7
	40.8 Ca	5.45	4.9	.55
	49.2 Ca	6.1	5.3	.8
	57.2 Ca	6.8	6.2	.6
	65.8 Ca	7.55	6.75	.8
	74.2 Ca	8.35	7.05	1.3

This means that there is an increased degree of buffering following drying. It is also evident that the drying has drawn the soil material together into larger and more definite aggregates than in the normal or moist state. This effect is, of course, most evident when well-dispersed colloidal materials are dried.

THE REVERSIBILITY OF pH CHANGES DUE TO DRYING

A number of replicate flasks of soil and base were dried and redispersed as indicated in the previous section. pH values were then determined on separate flasks in duplicate after different time intervals of soaking. The results, presented in table 4, show some tendency for the pH to increase in all cases, but it seems evident that the samples which have returned most quickly to about the same or to higher values than were obtained before drying are samples with no base added. In fact, all soils without base seem to have returned to essentially the original pH or higher in the time allowed, except the "muck." This is further illustrated by figures 1 and 2, which show a more complete recovery without extra base than with base.

It appears in table 4 that the various soils with base added may have reached a relatively stable pH level during the time required for the samples without base to return to the original values or higher. However, to compare the effect of much longer periods of soaking, both with dried and undried samples, several samples of 300 gm. each were placed in beakers and base solutions were added. After stirring at intervals for 2 days the pH was determined and each sample was divided into three parts. One series was then oven-dried for 2 days; a second series was kept moist continuously; and a third series was allowed to dry in the air with an occasional rewetting. pH values were then determined at intervals on small portions of soil paste removed from each beaker. The results (table 5) show that after sufficient continuous soaking time there was only a small difference in pH whether the soil was originally oven-dried, continuously wet, or alternately wet and air-dried during the entire period.

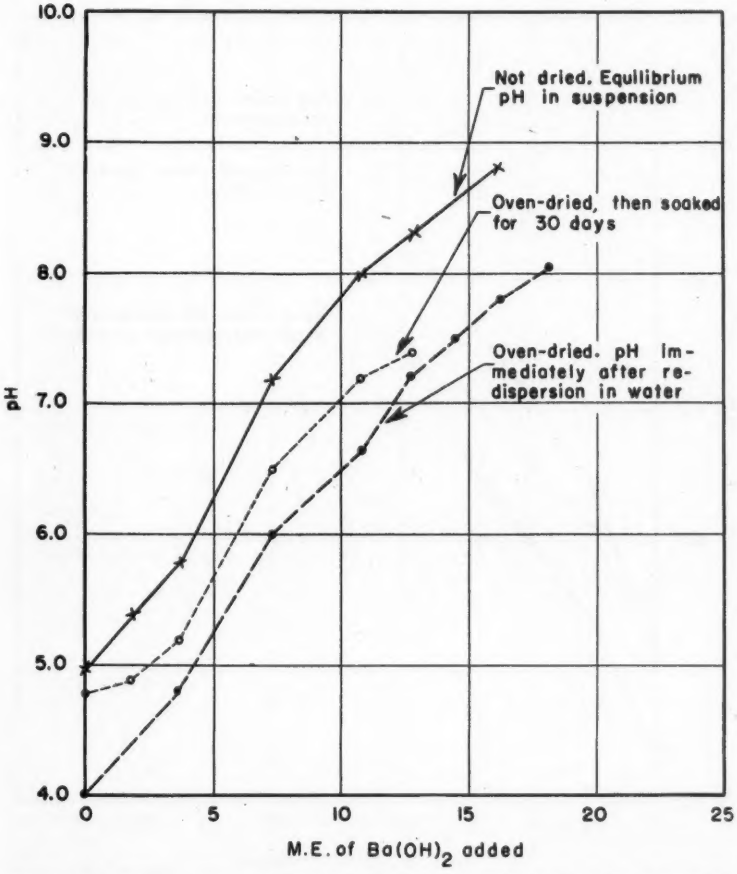

FIGURE 1.—Buffer curves of Zoar subsoil (soil No. 7a) showing the typical influence of drying and recovery after soaking. Note that the pH differences caused by drying are greater with base added than without and that the recovery by soaking is less complete.

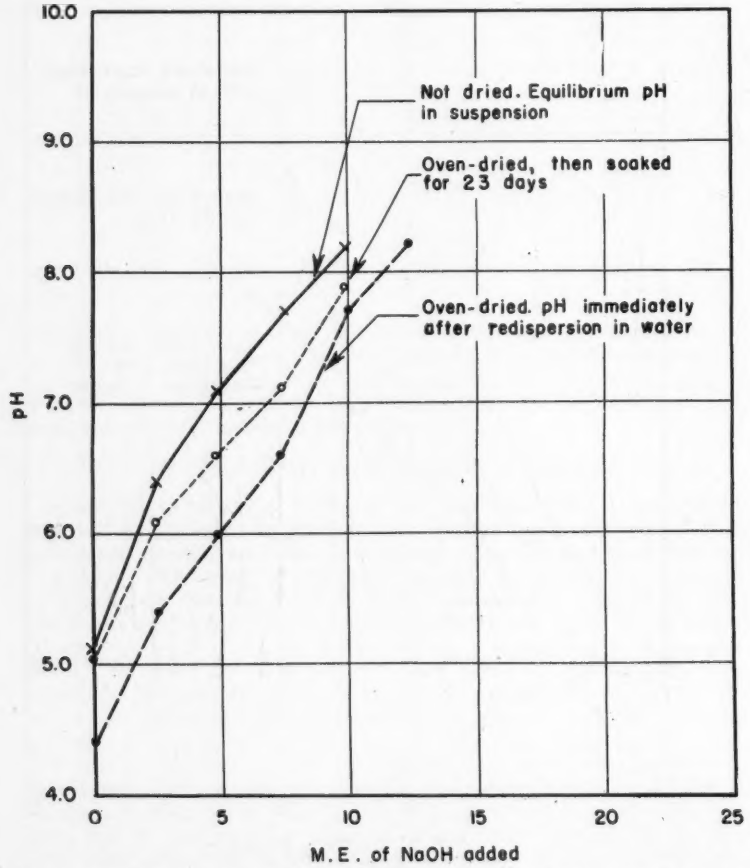

FIGURE 2.—Buffer curves of a Holston subsoil (soil No. 6a) showing the typical influence of drying and recovery after soaking. Note that the pH differences caused by drying are greater with base added than without and that the recovery by soaking is less complete.

TABLE 4.—*Reversibility of pH changes due to drying at 110° C.*

Dried, then soaked for time indicated	M. E. and kind of base per 100 gm. of soil		
	0	3.6 Ba	5.6 Ba
Soil 1:			
3 hours	4.2	4.8	5.1
6 days	4.4	5.4	
8 days	4.5	5.15	5.35
10 days	4.4	4.95	5.5
15 days	4.55	5.15	
22 days	4.75	5.25	5.55
Original value, undried	4.8	5.6	6.95
Soil 2:			
3 hours	4.9	5.9	
1 day			6.6
6 days	5.3	6.7	
8 days	5.4	6.5	
9 days			6.9
10 days	5.3	6.4	
15 days	5.5	6.5	
22 days	5.6	6.45	6.75
Original value, undried	5.3	6.9	7.1
Soil 5:			
3 hours	4.9	5.9	5.95
2 days			5.95
4 days			6.6
6 days	5.6	6.6	
8 days	5.55	6.35	
10 days		6.2	6.85
15 days		6.15	6.7
22 days	5.4	6.15	6.7
Original value, undried	5.2	6.65	7.05
Soil 8 (muck):			
3 hours	3.7	4.05	
6 days	3.9	4.65	
8 days	3.90	4.45	
10 days	3.95		
15 days	4.1	4.5	
22 days	4.3	4.7	
31 days	4.35	4.65	
Original value, undried	4.9	5.05	

	0	2.7 Ba	4.9 Ba	7.9 Ba	10.4 Ba
Soil 3:					
5 minutes	4.4	5.1	6.0	6.75	7.5
20 days	4.9	5.6	6.5	6.95	7.1
Original value, undried	5.0	6.2	6.75	7.3	7.8

	0	1.8 Ba	3.6 Ba
Soil 6:			
3 hours	4.65	5.0	6.2
6 days	4.75	5.9	6.85
10 days	4.85	5.8	6.85
14 days	4.85	5.9	6.85
21 days	5.0		
30 days	4.95	5.6	7.0
Original moist value	5.1	6.95	7.65

	0	1.8 Ba	3.6 Ba	7.2 Ba	12.7 Ba
Soil 7:					
5 minutes	4.0	4.35	4.8	5.9	7.1
3 hours	4.3	4.50	5.0	6.3	
6 days	4.3	4.55	5.15	6.5	7.4
10 days	4.5	4.75	5.10	6.6	7.5
14 days	4.5	4.85	5.10	6.35	7.3
21 days	4.5	4.65	5.10	6.5	7.4
30 days	4.75	4.90	5.15	6.5	7.4
Original moist value	4.9	5.4	5.75	7.2	8.3

TABLE 4.—*Reversibility of pH changes due to drying at 110° C.*—Continued

Dried, then soaked for time indicated	M. E. and kind af base per 100 gm. of soil.				
	0	3.6 Ba	7.2 Ba	10.8 Ba	12.6 Ba
Soil 7:					
5 minutes or less	4.0	4.8	5.95	6.60	7.20
2 days	4.4	4.95	6.27	6.85	7.40
5 days	4.75	5.20	6.3	6.75	7.1
13 days	4.5	4.90	6.1	6.6	7.0
21 days	4.65	5.05	6.25	6.7	7.05
35 days		4.8	6.1	6.7	7.2
Original value, undried	4.9	5.75	7.2	8.0	8.3
	0	1.9 Na	3.6 Na	7.8 Na	11.7 Na
Soil 7:					
5 minutes or less	3.95	4.4	5.1	5.9	7.2
2 days	4.2		5.2	6.2	7.25
5 days	4.55		5.55	6.35	7.4
13 days	4.55		5.3	6.0	7.1
21 days	4.55		5.55	6.45	7.5
35 days	4.5		5.5	6.5	7.4
Original value, undried	4.95	6.0	6.65	7.95	

Where the ultimate pH values are below 7 the difference due to drying is still probably significant even after 170 days of continuous soaking. If the drying had been continued for a longer period it is likely that the reversibility would have been somewhat less complete.

TABLE 5.—*Influence of long-continued soaking on the pH of several soils and subsoils which were oven-dried after an addition of base, compared to the same soils soaked continuously without drying and also the same kept alternately wet and air-dry*

Soil	M. E. of base	Treatment	Original pH of moist paste after 2 days with base	pH of samples after soaking—			
				30 minutes	43 days	127 days	171 days
Holston sandy loam	5.4 Ca	Oven-dry	7.9	6.8	7.3	7.8	7.6
	5.4 Ca	Continuously wet	7.9		7.4	7.6	7.5
	5.4 Ca	Alternately wet and air-dry.	7.9		7.2	7.5	7.6
Purdy silt loam	5.4 Ca	Oven-dry	6.4	5.35	6.35	7.3	7.0
	5.4 Ca	Wet	6.4		6.9	7.05	7.0
	5.4 Ca	Wet and air-dry	6.4		6.9	6.7	6.6
Dekalb clay subsoil	5.4 Ca	Oven-dry	5.3	4.4	4.6	4.9	5.0
	5.4 Ca	Wet	5.3		5.0	5.25	5.25
	5.4 Ca	Wet and air-dry	5.3		5.0	5.1	5.2
Cavode silty clay subsoil	5.4 Ca	Oven-dry	6.5	5.0	5.5	6.2	6.0
	5.4 Ca	Wet	6.5		6.2	6.4	6.4
	5.4 Ca	Wet and air-dry	6.5		6.1	6.2	6.1
Gilpin clay loam subsoil	5.4 Ca	Oven-dry	7.8	6.8	6.6	6.9	7.0
	5.4 Ca	Wet	7.8		7.05	7.05	7.05
	5.4 Ca	Wet and air-dry	7.8		7.15	7.35	7.5
Dekalb clay loam subsoil	4 Na	Oven-dry	5.7	4.8	5.2	5.45	5.7
	4 Na	Wet	5.7		5.5	5.7	5.8
	4 Na	Wet and air-dry	5.7		5.3	5.45	5.7

ALTERNATE SOAKING AND DRYING

Table 6 shows the results of alternate soaking and drying on the pH values of several soils without added base, and for one soil with several increments of Ba added. All differences shown are small and probably insignificant, indicating that the initial drying has given essentially the minimum pH value.

TABLE 6.—*Influence of alternate soaking and drying; each drying was for approximately 5 days*

Soil No.	M. E. of bases	pH of samples dried and soaked—		
		Once	Twice	Three times
1	None	4.3	4.2	4.15
2	do	4.9	4.85	4.8
3	do	5.1	5.05	5.0
5	do	4.9	4.95	5.0
7	do	4.0	4.05	4.0
8	do	3.85	3.80	3.85
		pH of sample soaked 30 days, then dried		
7	1.8 Ba	4.35	4.4	
	3.6 Ba	4.80	4.75	
	7.2 Ba	5.9	5.65	
	12.7 Ba	7.1	7.0	
	16.3 Ba	7.8	7.65	

INFLUENCE OF DRYING ON THE EXCHANGE CAPACITY AND RECOVERY OF ADDED BASES

Table 7 shows the exchange relations obtained on two soils with bases added and dried as described in previous sections. In the case of the surface soil it appears that a small amount of some bases, both Ba and Na, may have become fixed by the drying although most have been readily recovered by overnight leaching with the normal ammonium acetate. Recovery of bases seems essentially complete in the case of the clay subsoil. The base exchange capacity as measured by retention of the ammonium ion may be slightly decreased with

TABLE 7.—*Influence of oven drying upon the apparent exchange capacity and the recovery of added bases by neutral NH_4 acetate leaching*

SOIL 3—SILT LOAM SURFACE SOIL

Base added, M. E. per 100 gm.	Treatment after addition of base	Soil pH from buffer curve	Milliequivalents		Exchange bases, M. E.	Recovery of added bases		Bases not recovered, M. E.
			Exchange capacity	Apparent exchange H+		M. E.	Percent	
0	Air-dry	5.05	10.8	4.3	6.5			
0	Oven-dry	4.4	10.6	4.5	6.1			
2.5 Ba	do	5.05	9.5	1.8	7.7	1.6	64	0.9
3.8 Ba	do	5.5	10.3	1.0	9.3	3.2	84	.6
7.6 Ba	do	6.65	10.4		12.0	5.9	78	1.6
2.0 Na	do	5.2	10.0	2.6	7.4	1.3	65	.7
3.9 Na	do	5.75	10.0	.7	9.3	3.2	82	.7
7.8 Na	do	6.7	10.0		12.6	6.5	83	1.3

SOIL 7—CLAY SUBSOIL

0	Air-dry	5.0	20.5					
0	Oven-dry	4.0			15.6			
3.1 Ba	do	4.7	19.8	1.4	18.4	2.8	90	.3
4.7 Ba	do	5.1	21.6		21.1	5.5	115	
9.4 Ba	do	6.3	19.2		22.7	7.1	76	2.2
2.5 Na	do	4.7	19.5	1.2	18.3	2.7	108	
3.8 Na	do	5.0	20.2	.6	19.6	4.0	105	
7.6 Na	do	5.8	19.6		22.4	6.8	89	.84

both soils (excess ammonium acetate was removed by leaching with ethyl alcohol).

Much more detail about the relative degree of fixation of various ions by drying may be found in other work [3] (*23*). The data here are sufficient to show that drying may introduce certain complications into the measurements of exchange capacity and exchangeable bases.

INFLUENCE OF DRYING ON THE RELEASE OF H+ TO A NEUTRAL SALT

Successive additions of neutral normal $BaCl_2$ solution were shaken with two different soils to determine the release of H+. After shaking and centrifuging, the solution was poured off and back titrated to determine the release of H+. When an essential end

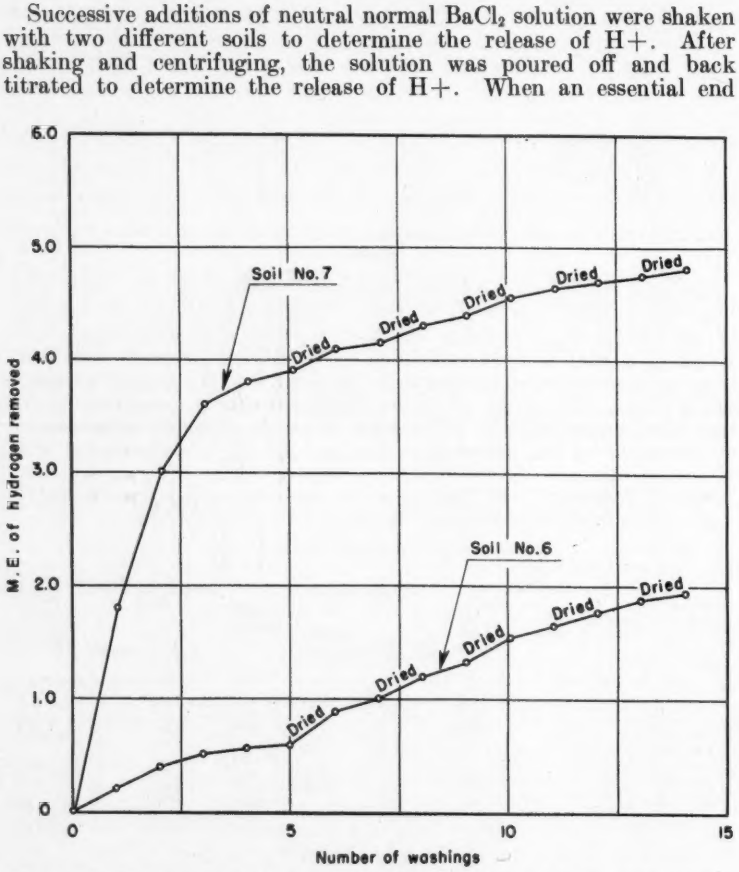

FIGURE 3.—Influence of drying on the release of H+ to a neutral salt solution of $BaCl_2$. Soil 7, a slack water clay subsoil, shows a distinct but small percentage increase in H+ release. Soil 6, a loam subsoil, shows a somewhat greater actual and a much greater percentage increase in H+ release.

[3] PAGE, J. B. THE RELATION OF IONIC SIZE TO THE FIXATION OF POTASSIUM AND OTHER CATIONS BY COLLOIDAL CLAY. Ohio State Univ. Doctoral Diss. 32. 1940.

point was approached after five washings, the soil and salt were oven-dried, redispersed, and the H+ titrated. As shown in figure 3, the successive dryings which followed caused an additional release of H+. The total and the percentage increase were much greater for the soil with lowest exchange capacity and less clay.

INFLUENCE OF DRYING ON SOIL BUFFERING AGAINST DIFFERENT CATIONS

It is evident from the tables that drying eliminates part of the differences in buffering commonly associated with the different cations. With Na the decrease in pH due to drying is consistently greater than with Ba. This would, of course, tend to bring Na and Ba buffer curves closer together because, as is well known, Na normally increases the pH of a soil more than an equivalent amount of Ba.

The drying influence on cation differences is shown more clearly for two soils in figures 4 and 5. Here the drying has been continued long enough to approach the minimum pH, which is not true of all the values shown in the tables.

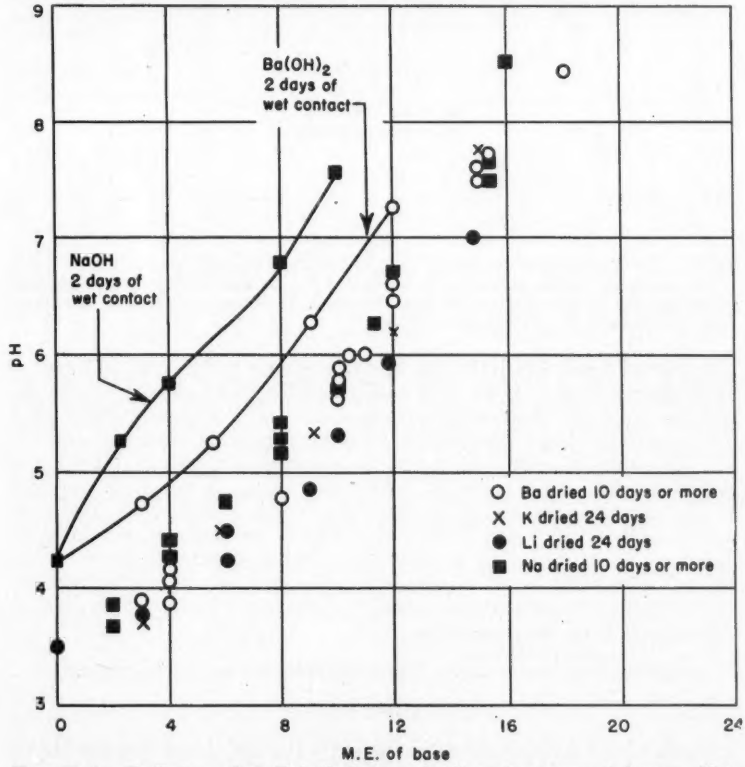

FIGURE 4.—Buffering of a Dekalb loam subsoil against several bases. Note the distinctly different curves for Ba and Na in moist suspensions but the overlapping of values after drying. It seems evident that drying eliminates much of the difference between bases.

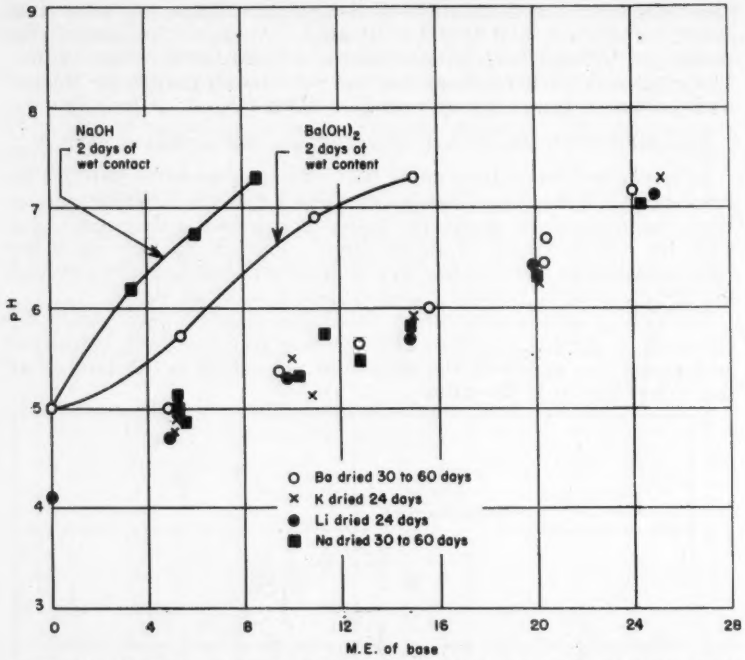

FIGURE 5.—Buffering of Purdy silt loam surface soil against several bases. Note the distinctly different curves for Ba and Na in moist suspensions but the overlapping of values after drying. Consistent differences between the bases do not seem to be evident after drying.

In figure 4 the soil buffering is dominated by the clay; in figure 5 by organic matter. With both soils the differences among Na, Li, K, and Ba are small and rather inconsistent. The writers suspect that the variations shown are mainly inherent errors, because 0.2 and 0.3 pH variations are common when an attempt is made to duplicate a particular dry buffer curve at a different date. Some of the sources of this error are shown to be associated with a lack of complete or equilibrium drying, some with the degree or time of grinding or shaking. In any case, it is clear that drying eliminates most of the differences among bases as they affect the pH of soil. This reduces soil buffering to a more or less absolute property which may be defined independently of the base used.

SOME FACTORS WHICH MAY CAUSE VARIATIONS IN THE DRYING EFFECT

DRYING TIME

Many of the data reported in the early part of this paper are based on a more or less indefinite time of drying. This appeared justified by the belief that a few days at 105° C. would invariably result in

constant weight and little likelihood of other changes. This assumption gave results that were satisfactory in showing some of the general influences of drying, but many unexpected and variable detailed results led to a more critical examination of the effect of time.

Table 8 shows that a few days of drying are not enough to give minimum pH values. There is a trend downward which extends over a period of 20, 30, or more days depending on the soil. When much organic matter is involved the time required to attain a minimum value seems to be longer than when the buffering material is clay.

TABLE 8.—*Influence of time of drying on pH of several different soils treated with bases; the bases were added in solution and drying was started after about 2 days of contact*

Soil	M. E. and kind of base	pH of soil after drying for number of days indicated												
		1	2	3	4	7	10	14	15	18	27	32	48	64
Ashby silt loam subsurface	11.0 Na	6.6	-----	6.45	6.2	5.8	6.0	5.8	-----	-----	-----	-----	-----	-----
	15.8 Na	7.6	-----	7.6	7.6	6.85	6.8	6.55	-----	-----	-----	-----	-----	-----
	14.0 Ba	7.0	-----	6.85	6.45	6.05	-----	-----	-----	-----	-----	-----	-----	-----
Calvin silt loam surface	4.9 Na	7.0	-----	6.8	6.35	5.5	5.55	5.5	-----	-----	-----	-----	-----	-----
	9.7 Na	7.9	-----	8.1	7.2	6.7	6.5	6.5	-----	-----	-----	-----	-----	-----
	3.5 Ba	6.2	-----	5.8	-----	5.4	-----	-----	-----	-----	-----	-----	-----	-----
	7.0 Ba	7.05	-----	6.9	-----	6.5	-----	-----	-----	-----	-----	-----	-----	-----
	10.5 Ba	7.9	-----	7.8	-----	7.5	-----	-----	-----	-----	-----	-----	-----	-----
Dekalb sandy clay loam subsoil	2.0 Na	-----	4.45	-----	-----	-----	4.0	-----	-----	3.87	3.95	-----	-----	-----
	6.0 Na	-----	5.45	-----	-----	-----	5.0	-----	-----	4.9	4.70	-----	-----	-----
	8.0 Na	-----	5.95	-----	-----	-----	5.75	-----	-----	5.5	5.3	-----	-----	-----
	2.9 Li	-----	-----	-----	-----	-----	-----	-----	3.8	-----	-----	3.7	3.9	3.7
	5.9 Li	-----	-----	-----	-----	-----	-----	-----	4.2	-----	-----	4.1	4.1	4.15
	11.9 Li	-----	-----	-----	-----	-----	-----	-----	6.0	-----	-----	5.9	6.1	5.85
	12.1 K	-----	-----	-----	-----	-----	-----	-----	6.7	-----	-----	6.4	6.6	6.05
Biago silt loam high organic	20 Na	-----	5.45	-----	4.9	-----	-----	-----	-----	-----	-----	-----	-----	-----
	50 Na	-----	6.95	-----	6.4	-----	-----	-----	-----	-----	-----	-----	-----	-----
	70 Ba	-----	7.05	-----	6.7	-----	-----	-----	-----	-----	-----	-----	-----	-----
Purdy silty clay	4.9 Li	-----	-----	-----	-----	-----	-----	-----	5.1	-----	-----	4.8	5.0	4.7
	24.7 Li	-----	-----	-----	-----	-----	-----	-----	7.7	-----	-----	7.2	7.45	7.1
	5.0 K	-----	-----	-----	-----	-----	-----	-----	5.1	-----	-----	4.95	5.0	4.8
	15.0 K	-----	-----	-----	-----	-----	-----	-----	6.6	-----	-----	6.1	6.6	5.9

The complete explanation of this long time requirement is somewhat uncertain, but it may be related to the loss of the last remnants of water from the sample. Table 9 shows that a significant loss of weight by the type of samples being studied extends over a considerable period of time. This water would, of course, be concentrated on colloidal surfaces as would the various cations and might thus exert an influence out of all proportion to the quantity of water involved.

TABLE 9.—*Influence of time of drying on loss of weight by 20-gram samples of soil treated with bases*

Soil	M. E. of base	Cumulative percent loss of weight by oven drying for number of days indicated					
		2	4	8	16	24	36
Dekalb loam subsoil	15 Na	Start	0.028	0.061	0.127	0.125	0.127
	12 K	do	.038	.102	.149	.150	.149
Purdy silt loam surface	20 Na	do	.183	.400	.700	.811	.902
	17 K	do	.177	.403	.767	1.090	1.196

Grinding

It has not been considered desirable to grind samples for the pH determinations, but tests were made to find out whether this might be a source of error. Table 10 shows that grinding to pass a 40-mesh screen can cause a definite change of pH. The effect may be either an increase or a decrease depending on the nature of the soil (*2*). Grinding should, therefore, be avoided.

Table 10.—*Changes in pH of oven-dried samples caused by grinding*

Soil	Base M. E.	pH of slaked samples	pH of ground samples	Change due to grinding
Dekalb sandy clay loam subsoil	12 Na	7.85	8.1	+0.25
	10 Na	6.6	6.9	+.30
	8 Na	5.95	5.95	None
	6 Na	5.45	5.15	−.30
	4 Na	4.65	4.9	+.25
	2 Na	4.45	4.6	+.15
	14 Ba	7.95	7.75	−.20
	12 Ba	7.3	7.15	−.15
	10 Ba	6.3	6.1	−.20
	8 Ba	5.2	5.25	+.05
	6 Ba	4.75	4.8	+.05
	4 Ba	4.45	4.45	None
	3 Ba	4.2	4.3	+.10
Blago silt loam	50 Na	7.5	6.95	−.55
	40 Na	7.1	6.5	−.60
	30 Na	6.35	5.8	−.55
	20 Na	5.65	5.45	−.20
	80 Ba	7.6	7.3	−.30
	70 Ba	7.3	7.05	−.25
	60 Ba	6.85	6.05	−.80
	40 Ba	5.8	5.7	−.10
	30 Ba	5.35	4.75	−.60
	20 Ba	4.85	4.25	−.60
	10 Ba	4.0		

Time of Crushing

Although thorough grinding is unnecessary, a sample dried from a suspension must be crushed in order to give a good mixture and to be suitable for the pH test. It did not seem that the exact procedure for crushing should make much difference, but comparisons shown in table 11 indicate that the time of crushing can influence the results.

Table 11.—*Influence of time of crushing on the pH of Dekalb sandy clay loam subsoil dried with different increments of base*

Base M. E.	pH of soil paste			
	Crushed after 1 day, then dried for—		Crushed only after drying for—	
	30 days	44 days	31 days	45 days
3.0 Na	4.5	4.5	4.6	4.8
7.1 Na	5.3	5.2	5.3	5.3
11.1 Na	6.45	6.3	6.8	6.55
15.2 Na	8.3	8.1	8.4	8.3
2.95 K	4.6	4.25	4.4	4.55
7.1 K	4.9	4.8	5.2	4.95
10.9 K	5.9	5.8	6.1	6.0
15.1 K	7.9	7.8	8.4	8.4

For some reason, crushing after 1 day of drying resulted in lower values than when the soil mass remained intact until the end of an extended drying period.

Temperature of Drying

Since drying at a fixed temperature such as 105° C. is somewhat arbitrary, a higher temperature was used with some samples to determine whether this might cause an even lower pH. But the differences in pH values at 150° and 105° were rather small and inconsistent. In some cases a lower value was obtained, in others a higher one. A few comparisons are summarized in table 12. It appears that with sufficient time a true minimum is reached at about 105°, and the 150° temperature cannot be expected to cause any additional lowering of the pH. A small increase in pH with some samples appears significant and may be due to the volatilization of certain acid constituents at the 150° temperature.

Table 12.—*A comparison of the pH values obtained after drying at 105° C. and at 150° C.*

Soil	Amount and kind of base added, M. E.	pH after drying at—			
		105° C.		150° C.	
		15 days	Approximately 5 days	28 days	Approximately 5 days
Dekalb sandy clay loam	3 K	3.8		4.5	
	6 K	4.5		4.8	
	9 K	5.4		5.4	
	12 K	6.6		6.5	
	15 K	7.9		8.0	
	3 Na	3.8		4.4	
	11.2 Na	6.0		6.2	
Litz silt loam surface	None		5.2		5.3
Do	do		5.5		5.5
Frederick subsurface	do		6.0		5.9
Dekalb sand	do		4.0		4.2
Calvin subsurface	do		4.0		4.1
Calvin subsoil	do		3.6		3.7
Ashby silt loam surface	do		4.1		4.1
Ashby silt loam subsurface	do		4.0		4.1
Do	do		4.0		4.1
Corydon clay subsoil	do		6.9		6.0
Ungers silt loam surface	do		4.4		4.7
Ungers silt loam subsoil	do		4.5		4.5

GENERAL DISCUSSION

Results presented show that with the varied soils and soil materials studied drying causes a considerable change in soil buffering relationships, as well as in the observable aggregation. It appears that the buffer curve of any soil may be thought of as being defined within two extremes. The weakest buffering is that obtained by placing a soil and a base in contact in a dilute suspension. After approximately 2 days of contact a fairly well defined curve is obtained which is ordinarily accepted as the buffer curve for the soil with the particular base used. This is also ordinarily the most dispersed condition for the soil. The curve is invariably higher on the pH scale for bases

like lithium and sodium hydroxides than for calcium or barium. This also corresponds with the degree of dispersion.

If, after essential equilibrium has been attained, the soil-base suspension is thoroughly dried, then redispersed in water, a different buffer curve is obtained. The soil is also more completely aggregated. This curve invariably falls completely below the ordinary curve on the pH scale and shows stronger buffering. The least actual pH change caused by the drying is usually for the soil with no base added. Here, too, there is likely to be the least difference in aggregation.

Soaking in water following drying causes increases in the pH values. It also causes the soil to tend to redisperse. The soil with no extra base added usually returns to essentially the original value within 30 days, but with base additions the pH may not entirely return even after 6 months of continuous soaking; that is, the most quickly reversible process seems to be that which involves the soil without freshly added base. Drying apparently changes some relation between the soil and fresh base in such a way that it is only very slowly and often incompletely reversible. This change would seem to consist of a closer association of the basic ion with the exchange complex much the same as aggregation is favored by the drawing together of the colloidal surfaces. A closer attachment would naturally be expected as a result of drying, and it also seems reasonable that a hysteretic effect might result from drying, as in many physical and chemical phenomena. This might be thought of as a simple mechanical entrapment. The extreme smallness of the H+ ion when dehydrated would explain why it might completely escape entrapment and be free to contribute to a somewhat increased concentration of hydrogen ion, or lower pH. In the case of base-free soil (acid leached or electrodialized) the effect of drying on pH might be accounted for by a drawing together of colloidal surfaces so closely that some negative charges would become ineffective. Rehydration by soaking would reverse this process, as shown by the increase of pH values.

When soils are limed in the field the process of aging, primarily wetting and drying, would bring bases into a closer association with colloidal surfaces just as indicated in the laboratory. This would help to account for the fact that a liming factor has been necessary to convert laboratory buffer values into field liming recommendations. The dry buffer curve as described gives an indicated buffering which is of the proper order of magnitude to account for a liming factor of 1.5 to 2.0. It seems likely that many of the recognized variations of soil pH in the field both before and after liming are within the zone defined by the moist suspension and the dry buffer curve. The momentary pH would be expected to reflect the relationship of the soil and base with respect to the cycle of hydration or dehydration. The lowest pH would be the point of closest attachment between soil and base, and also between colloidal surfaces. It would, therefore, seem to correspond to the highest aggregation.

Since drying completely alters the shape of a soil buffer curve, giving essentially a straight line relationship between pH and milliequivalents of base added in many cases, there seems to be some doubt as to whether the shape of the moist buffer curve is very significant in determining such properties as exchange capacity. The shape changes

again on hydration but does not seem to have a well-defined form which can be readily interpreted.

Drying was also shown to influence the amount of exchangeable H+ as determined with a neutral salt ($BaCl_2$). With neutral ammonium acetate leaching it was shown that for two soils the retention of NH_4 ions as a measure of exchange capacity was little altered by drying. The bases recovered were sometimes in excess of the NH_4+ retention, although the pH of the soils with bases was well below 7. Drying thus caused an apparent contradiction by this method of determination. It seems likely that other methods of determining exchangeable H+, exchangeable bases, and exchange capacity might be influenced in a manner similar to that shown for the buffer curve, the $BaCl_2$, and the NH_4Ac methods. A consideration of the hydration factor and its influence on the tightness with which bases are held might help to reconcile some of the discrepancies among methods and explanations noted in the literature. An apparent build-up of exchange, for instance, could be accounted for by a closer association between the cation and the colloid, at least by certain methods of determination.

The fact that the drying effect upon soil pH and buffering is similar for a variety of soils and subsoils, for one electrodialyzed clay with high exchange capacity, for pure kaolinite and for bentonite seems to indicate that the phenomenon is of very general application. This is further emphasized by the comparisons among bases which show that Na, Ba, K, Li, and Ca all respond similarly. These similarities among soils and among the bases suggest that the drying influence can be classed as a typical hysteresis loop in buffer curves, and is probably traceable to some sort of closer association between cation and colloidal charges.

There are definite indications from observations and from actual measurements that drying increases soil aggregate stability (*16*) as well as acidity. This aggregation may be related to the indicated drawing together of clay surfaces and increases in acidity. At least, this should be investigated further along with the detailed physical-chemical relationships mentioned. This suggests that the aggregation and the increased acidity may be related through a common cause. The cause which appears most likely is the drawing of colloidal surfaces so close together by drying that molecular forces prevail and tend to prevent a complete rehydration. This paper does not offer an answer to many of the detailed, quantitative questions which are raised in considering the effect of drying on soil acidity and aggregation. Much more experimental work and theoretical study appear needed. The interrelation of drying, hydration, base exchange, acidity, and soil aggregation seem to appeal almost equally to the theoretical and the practical soil scientist.

SUMMARY

A review of the literature suggests that many questions regarding soil buffering relationships are not yet answered. The effect of drying is introduced as one approach to answering some of the theoretical and practical questions involved.

pH determinations of many varied samples show that oven drying

definitely increases the acidity as compared to that of moist or air-dried materials. The effect is most pronounced when freshly added bases are involved but is also evident in soils leached free of bases. An increased buffering results from the greater effect with added bases. Alternate soaking and drying has little more effect than a single drying. The reversibility of the effect of drying with fresh bases is very slow and probably never complete, at least with some soils. When no new bases are added the pH usually returns to the original value in 30 days or less.

Oven drying had only a small influence on the exchange capacity or exchangeable bases in one surface silt loam and one clay subsoil by ammonium acetate leaching; but it resulted in the peculiar situation of having a higher content of bases than the exchange capacity held by the soil at pH values well below neutrality. Oven drying of soils also increased the release of H+ to a neutral salt.

Buffering comparisons involving Li, Na, K, Ba, and Ca show that drying eliminates most if not all of the differences normally shown by these different cations.

A consideration of factors causing variations in the effect of drying shows that drying of 20, 30, or even more days is necessary to give a minimum, reproduceable pH. Grinding after drying increased the pH in all cases with a subsoil clay and decreased it with a high organic surface soil. Crushing in the early stage of drying caused the pH to go somewhat lower than when crushing was delayed until after a prolonged drying. A temperature of 150° C. gave results only slightly different from those at 105°, and in most cases the change was a small increase in pH.

A general discussion points out that the drying effect may help to reconcile some apparent contradictions of methods and results in the literature. It also appears to have possible application both in practical and in theoretical studies. The similarity of results with the various cations offers some opportunities for establishing an absolute buffering value independently of the cation used. In field liming the indicated dry buffering may help to account for the necessity of a "liming factor" to convert laboratory values to field requirements.

The fact that aggregation and acidity are both increased by drying suggests that the drawing together of colloidal surfaces may be the fundamental cause of both phenomena. Much more study is needed to clarify this point and other details of the interrelations among drying and rehydration, buffering, and aggregation.

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HATCHABILITY IN RHODE ISLAND REDS AS AFFECTED BY AGE OF PARENTS[1]

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INTRODUCTION

Hatchability in individual females or in pens is usually measured by the percentage of fertile eggs that hatch. This character is vitally important in the economical propagation of the flock. Females that give low hatchability have very little value as reproducers and are usually discarded even though they may possess many desirable characters as far as egg production is concerned. There is rather general agreement among investigators that hatchability is an individual trait and that both heredity and environment have important effects.

Landauer (*10*)[2] in 1941 reviewed the problem thoroughly so that any extensive review of the literature is unnecessary. Insko, Steele, and Wightman (*9*) have brought the literature up to date (1947) and have presented new data pointing to a decline in hatchability as females grow older.

EXPERIMENTAL RESULTS

The hatching records of pedigreed Rhode Island Reds bred at the Massachusetts Agricultural Experiment Station for high fecundity have been examined for the 15-year period from 1933 to 1947. Only individuals laying 10 or more fertile eggs have been included. It is the opinion of the writers that a considerable volume of data secured over a period of time presents a truer picture of the actual than would be the case with *fewer data subjected* to detailed statistical treatment.

AGE OF MALE IN RELATION TO HATCHABILITY

Male breeders ranged in age from about 10 months to 48 months. Males were grouped into four classes: cockerels (up to 12 months); yearlings (up to 24 months); 2-year-olds (up to 36 months); and 3-year-olds (48 months +). Most of these males were mated to females of various age ranges; thus the relation of age of male to hatchability may be examined.

The data show that the mean hatchability of the four classes was as follows: 135 cockerels, 83.64 percent; 143 yearlings, 80.67 percent; 60 2-year-olds, 82.26 percent; and 12 3-year-olds, 80.67 percent.

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[2] Italic numbers in parentheses refer to Literature Cited, p. 290.

These gross data suggest that the age of male breeders has very little, if any, effect on hatchability

A more accurate measure of the effect of age of males lies in the comparison of the records of identical males in successive years not shown in tables. These males were mated to similar age groups of females. Eight males which in the breeding pens had a mean hatchability of 84.6 percent as cockerels had a mean hatchability of 81.2 percent as yearlings. Five males were used in 3 successive years with the following hatchability records: As cockerels, 86.3 percent; as yearlings, 73.5 percent; and as two-year-olds, 90.2 percent. Seventeen males placed in the breeding pens first as yearlings gave a mean hatchability of 83.3 percent this first year, 79.0 percent the next year. Four males were tested first as yearlings and for the 2 following years. Their hatchability records were 89.8, 84.3, and 82.6 percent.

These rather limited data suggest that hatchability declines at a very slow rate as males grow older. This fact is in accord with previous observations (*5*, *11*, *4*, *6*) that hatchability is governed largely by inheritance which should not change with age, but that certain unknown physiological factors may also be in operation.

The complete data for the 15-year period are presented in table 1.

TABLE 1.—*Hatchability and age of breeders for the 15-year period*

Mating	Sires	Dams	Average hatchability
	Number	*Number*	*Percent*
Pullet ×:			
Cockerel	87	518	83.8
Yearling	43	234	81.9
2-year-old	16	66	82.7
3-year-old or older	2	3	90.1
Totals and means	148	821	83.2±0.8607
Yearling ×:			
Cockerel	26	97	84.8
Yearling	69	342	81.5
2-year-old	25	114	84.9
3-year-old or older	5	17	83.3
Totals and means	125	570	82.9±0.8842
2-year-old ×:			
Cockerel	17	42	83.4
Yearling	20	48	78.5
2-year-old	12	36	80.9
3-year old or older	5	17	74.2
Totals and means	54	143	80.2±1.9542
3-year-old or older ×:			
Cockerel	5	8	75.5
Yearling	11	15	74.5
2-year-old	7	10	74.1
3-year-old or older			
Totals and means	23	33	74.6±2.4902

AGE OF FEMALES IN RELATION TO HATCHABILITY

The means for the four age groups of females are given together with their standard error. It will be observed that a significant drop in hatchability first appeared in females that were 3 years old or older. Since there were only 33 females in this oldest group the decline in the mean does not afford conclusive evidence.

THE CHANGE WITH AGE IN THE HATCHABILITY OF IDENTICAL FEMALES

A total of 131 females gave a mean hatchability of 87.6 percent in their pullet year and 81.8 percent in their yearling year. Of 25 females used in 3 successive years, the mean hatchability was 91.8 as pullets, 84.3 as yearlings, and 83.0 percent as 2-year-olds. Seventy females were first placed in the mating pens as yearlings and were again used for breeding when 2-year-olds. Their hatchability as yearlings was 87.1 percent and as 2-year-olds, 77.9 percent. Twenty females that began their test as yearlings were tested over 3 years with a mean hatchability of 93.0 percent, 87.0 percent, and 74.4 percent.

In general, there was more decline in hatchability as age increased when identical females were examined than was shown in table 1 for the whole female population. This difference would be expected because a considerable number of pullet breeders gave low hatching records which eliminated them from further testing and reduced the mean for the complete pullet group. This same situation also applies to females that began their test as yearlings. In general, these data indicate that pullet or yearling breeders are likely to show a decline in hatchability with age.

IDENTICAL MATINGS IN SUCCESSIVE YEARS

Few identical matings are available for study of the effect of age of both parents on hatchability. Five identical matings were used in which the same parents were placed in the mating pens as cockerels and pullets and again as yearlings. The mean hatchability in these cases was 81.8 percent for the first year and 76.1 percent for the second year.

Five matings in which the sires were 2-year-olds and the dams were yearlings in the first year and in the second year both parents were 1 year older gave means of 93.6 percent and 80.1 percent hatchability respectively. These limited data from identical matings indicate something of a tendency for hatchability to decline as parents grow older.

EMBRYONIC MORTALITY IN RELATION TO AGE OF PARENTS

Dead embryos were grouped into four general classes with respect to age at death. Early deaths include those from the first to about the fifth day of development; late deaths include those from about the sixth to the eighteenth day; and very late deaths those that occurred after the eighteenth day up to full term. Very late live embryos include those developed to full term, remaining alive on the twenty-second day but failing to emerge from the shell.

Early embryonic mortality was practically the same from pullet and yearling mothers, about 4.3 percent (table 2). This means that slightly more than 4 percent of the fertile eggs gave rise to embryos that died early in incubation. There was some increase in mortality of embryos from 2-year-old mothers and a very marked increase in those from the very old hens. Table 2 shows also that this tendency for embryos to die early generally increased when the older males were mated to 2-year-old or older hens. Any effect of age of male does not appear when pullet or yearling mothers were used.

TABLE 2.—*Percentage of embryonic mortality for the 15-year period*

Mating	Sires	Dams	Average embryonic mortality		
			Early	Late	Very late [1]
Pullet ×:	*Number*	*Number*	*Percent*	*Percent*	*Percent*
Cockerel	87	518	4.1	9.4	2.3
Yearling	43	234	4.7	10.4	2.2
2-year-old	16	66	5.0	10.6	1.3
3-year-old or older	2	3	1.1	4.7	2.0
Totals and means	148	821	4.3±0.2735	9.7±0.6397	2.2±0.1903
Yearling ×:					
Cockerel	26	97	2.8	8.1	2.9
Yearling	69	342	5.1	9.8	3.1
2-year-old	25	114	4.0	7.7	1.8
3-year-old or older	5	17	4.6	8.4	2.8
Totals and means	125	570	4.4±0.3396	9.0±0.5325	2.8±0.2896
2-year-old ×:					
Cockerel	17	42	4.5	7.7	3.7
Yearling	20	48	5.0	13.5	2.7
2-year-old	12	36	9.7	7.3	1.0
3-year-old or older	5	17	9.1	12.2	2.9
Totals and means	54	143	6.3±0.6690	10.2±1.1762	2.7±0.5003
3-year-old or older ×:					
Cockerel	5	8	7.1	12.2	3.2
Yearling	11	15	9.0	12.8	2.1
2-year-old	7	10	12.9	8.5	3.9
3-year-old or older	0	0			
Totals and means	23	33	9.8±1.7205	11.4±1.8938	2.9±0.6945

[1] Fully developed embryos that may or may not break the shell.

There was no evidence to indicate any significant effect of age of either parent on late or on very late embryonic death rate.

FULL-TERM LIVE EMBRYOS THAT FAIL TO EMERGE

Specific causes for the failure of live embryos to emerge from the shell are but little understood. A small number of lethals that produce morphological effects have been described, and the influence of position has been extensively studied by Byerly and Olsen (*2*), Hutt (*7*), Insko, and Martin (*8*), and many others. The work of Byerly and Olsen (*1*), Dove (*3*), and Waters (*12*) raises doubt concerning the importance of malpositions as factors in hatchability.

Table 3 gives the complete data on the percentage of full-term embryos that failed to emerge even though the embryos were alive on the twenty-second day. The relative incidence ranges from 1 to 9 percent, but the average is rather low. There is considerable evidence to suggest that when the parents are older, the incidence is higher. This fact accounts in part for the decline in hatchability with increase in age of parents.

The general results of the study of embryonic mortality over 15 years seem to point to a higher early embryonic death rate from matings of older parents, to no significant relation of age of parents to late or very late embryonic death rate, and to an increase in the percentage of live embryos that fail to emerge in eggs from older parents. These facts largely explain the observed decline in hatchability as birds grow older, but it seems probable that unrecognized physiological factors also are operating.

TABLE 3.—*Percentage of live full-term embryos that failed to emerge during the 15-year period*

Mating	Sires	Dams	Average
Pullet ×:	*Number*	*Number*	
Cockerel	30	202	1.4
Yearling	17	124	2.0
2-year-old	7	30	1.0
3-year-old or older	2	3	2.2
Totals and means	56	359	1.5±0.2012
Yearlings ×:			
Cockerel	8	27	4.6
Yearling	22	125	1.9
2-year-old	12	47	3.3
3-year-old or older	2	4	2.4
Totals and means	44	203	2.8±0.3954
2-year-old ×:			
Cockerel	6	13	2.0
Yearling	3	7	2.1
2-year-old	7	24	1.9
3-year-old or older	2	7	4.0
Totals and means	18	51	2.2±0.5911
3-year-old or older ×:			
Cockerel	3	5	3.4
Yearling	2	3	9.0
2-year-old	3	4	1.7
3-year-old or older			
Totals and means	8	12	4.2±1.6503

SUMMARY

A study was made of the complete hatching records of Rhode Island Red chickens bred for high fecundity over a 15-year period to discover possible relations between age of parents and hatchability. The data included 135 cockerels, 143 yearlings, 60 2-year-olds and 12 3-year-olds as male parents, and 821 pullets, 570 yearlings, 143 2-year-olds, and 33 older hens as female parents.

The following deductions were made from these data:

(1) Hatchability declines at a slow rate as males grow older and at a somewhat higher rate as females grow older.

(2) Males used in successive years exhibit some decline in hatchability.

(3) Females tested in successive years tend to show a declining hatchability.

(4) Identical matings in successive years demonstrate this effect of age.

(5) Embryonic death records show a greater incidence of early embryonic deaths from eggs of older parents as well as a higher incidence of full-term live embryos that failed to emerge.

(6) Late and very late embryonic deaths were no higher in eggs from older than from younger parents.

(7) No specific causes for the changes in embryonic losses with increased age of parents have been found.

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EFFECT OF SODIUM SULFAMERAZINE ON SALMONELLA PULLORUM[1]

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INTRODUCTION

The therapeutic value of some of the sulfonamides in reducing mortality among chicks infected with *Salmonella pullorum* has been shown by the work of Severens, Roberts, and Card,[3] by Roberts, Card, and Alberts,[4] and by others. It is well known that in some cases a tolerance to a sulfonamide may be acquired by bacteria. It is therefore important to know whether or not *Salmonella pullorum* may acquire tolerance, for if it does, the effective use of sulfonamides in the control of pullorum disease may be greatly decreased. A culture of *Salmonella pullorum* was grown in increasing concentrations of sodium sulfamerazine and chicks were inoculated with the treated and untreated cultures to determine what effect, if any, was produced by the sodium sulfamerazine.

METHODS

A culture was produced from a single bacterium. This was divided into two subcultures. One served as the control and the other was grown in a medium containing sodium sulfamerazine. The basic medium used was tryptose phosphate broth with 0.2 percent dextrose. The beginning concentration was 100 mg. of sodium sulfamerazine per 100 ml. of medium. This was increased by 10-mg. increments until the final concentration of 200 mg. was reached. The length of time between the first and final concentration was 98 days. The first inoculations of chicks with the treated and control cultures were made when the culture had been grown for 36 days in a 200-mg. concentration of sodium sulfamerazine (table 1). At the second and third inoculations the times were 268 and 275 days respectively (table 2). At the last inoculation the time was 298 days (table 3). All chicks were New Hampshires and were from pullorum-free flocks. During the first 134 days transfers were made at least daily and after that about twice each week. The control or untreated culture was transferred at the same time as the treated culture, so that at any given time the number of transfers was the same for both cultures. When transfers were first made to a higher concentration an inhibiting effect on growth occurred. This soon disappeared and in the highest

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[2] Acknowledgment is made to Sharp and Dohme, Inc., for financial aid in this investigation.

[3] SEVERENS, J. M., ROBERTS, E. and CARD, L. E. THE EFFECT OF SULFONAMIDES IN REDUCING MORTALITY FROM PULLORUM DISEASE IN THE DOMESTIC FOWL. Poultry Sci. 24: 155–158. 1945.

[4] ROBERTS, E., CARD, L. E. and ALBERTS, J. O. THE USE OF SULFONAMIDES IN THE CONTROL OF PULLORUM DISEASE. Poultry Sci. 27: 194–200. 1948.

concentration of 200 mg. per 100 ml. of medium the growth was finally as great as that of the untreated culture in normal medium. This indicates an acquisition of tolerance to sodium sulfamerazine by the treated culture. Attempts to use a higher concentration always resulted in marked inhibition of growth.

When the untreated culture was put in the 200-mg. concentration of sodium sulfamerazine, inhibition of growth occurred but it was not lethal. A concentration of 1,000 mg. per 100 ml. was lethal, but the pH was above 11 and the lethal effect may have been due to alkalinity rather than to the sodium sulfamerazine.

It should be pointed out that the concentration of sodium sulfamerazine may have changed as the bacteria increased in number. As they grew, acid was produced which lowered the pH, and some precipitation of sulfamerazine resulted. All attempts to buffer the medium failed to prevent precipitation.

All cultures were incubated at 37° C. and examined after 12 and 24 hours. The degree of growth in cultures was observed by photonephelometer turbidity readings and by plate counts. For inoculation, both treated and untreated cultures were standardized to contain the same number of bacteria per dose. Sodium sulfamerazine was administered in the feed beginning when the chicks were 1 day of age.

RESULTS

The first test of untreated and treated cultures was made with day-old chicks orally inoculated with ¼ ml. of 24-hour cultures of *Salmonella pullorum*. All the chicks were fed the same ration containing 0.5 percent sodium sulfamerazine. The treated culture had been grown in the final concentration of 200 mg. of sodium sulfamerazine in 100 ml. of medium 36 days before the inoculation of chicks. The percent mortality during the 21-day period was 29.4 among the chicks inoculated with the untreated culture and 13.7 for the group inoculated with the treated culture (table 1).

TABLE 1.—*Mortality of chicks orally inoculated at 1 day of age with untreated and treated* [1] *cultures*

Chicks (number)	Inoculated with culture—	Ration No.	Mortality in 21 days	Average weight of survivors at 21 days
			Percent	*Grams*
51	Untreated	391 +0.5 percent Ssm [2]	29.4	118
51	Treated	do	13.7	135

[1] Grown in medium with a final concentration of 200 mg. of sodium sulfamerazine in 100 ml. for 36 days
[2] Sodium sulfamerazine.

The treated culture was continued in the 200-mg. concentration medium and the second and third tests were made 232 and 239 days later. In these tests the chicks were subcutaneously inoculated at 3 days of age with ⅒ ml. of 24-hour cultures. In addition to the two groups of chicks that received 0.5 percent sodium sulfamerazine in the feed (ration 391), two other groups were inoculated which did not receive sodium sulfamerazine in the feed. The results are given in table 2. Mortality was significantly greater ($P<0.01$) among

TABLE 2.—*Mortality of chicks subcutaneously inoculated at 3 days of age with untreated and treated cultures*

Chicks (number)	Inoculated with culture—	Ration No.	Mortality in 21 days	Average weight of survivors at 21 days
			Percent	*Grams*
52	Untreated	391	53.8	112
49	do	391	75.5	107
101			64.4	110
52	Treated [1]	391	98.1	94
50	do [2]	391	92.0	85
102			95.1	8
52	Untreated	391 +0.5 percent Ssm	36.5	120
50	do	do	54.0	117
102			45.1	118.8
51	Treated [1]	391 +0.5 percent Ssm	60.8	107
49	do [2]	do	57.1	96
100			59.0	101.4

[1] Grown in medium with 200 mg. of sodium sulfamerazine per 100 ml. for 268 days.
[2] Grown in medium with 200 mg. of sodium sulfamerazine per 100 ml. for 275 days.

chicks inoculated with the culture which had been grown in the presence of sodium sulfamerazine regardless of whether the chicks were given sodium sulfamerazine in the feed.

In a fourth series of tests made 23 days after the third test, but using ration 483, similar results were obtained among chicks not protected by sodium sulfamerazine. With the untreated culture the mortality was 32.4 percent and with the treated culture it was 61.8 percent (table 3). This difference in mortality is significant ($P<0.01$). These mortalities were lower than those on ration 391, which were 64.4 percent and 95.1 percent for untreated and treated cultures, respectively (table 2). The chicks referred to in table 2 were inocu-

TABLE 3.—*Mortality of chicks orally inoculated at 1 day of age with untreated and treated [1] cultures*

Chicks (number)	Inoculated with culture—	Ration No.	Mortality in 21 days	Average weight of survivors at 21 days
			Percent	*Grams*
51	Untreated	483	23.5	124
51	do	483	41.2	128
102			32.4	125.7
51	Treated	483	66.7	110
51	do	483	56.9	99
102			61.8	103.8
51	Untreated	483+0.5 percent Ssm	3.9	145
51	do	do	11.8	143
102			7.8	144
49	Treated	483+0.5 percent Ssm	10.2	139
49	do	do	4.1	129
98			7.1	133.3

[1] Grown in medium with 200 mg. of sodium sulfamerazine per 100 ml. for 298 days.

lated subcutaneously and those referred to in table 3 were inoculated orally. It has been found by Severens, Roberts, and Card,[5] that mortality among chicks subcutaneously inoculated at 3 days of age does not differ greatly from the mortality from oral inoculation at 1 day of age. The differences in mortality were about the same, 30.7 percent on ration 391 and 29.4 percent on ration 483. In the last test when the inoculated chicks on ration 483 were protected by 0.5 percent sodium sulfamerazine in the feed mortality was practically the same, being 7.8 percent for the untreated culture and 7.1 percent for the treated culture. A suggested explanation for this is that the combined protection of ration 483 and the sodium sulfamerazine was sufficient to reduce mortality to a very low level. Tests are now under way to determine the effect of several different rations on mortality among chicks inoculated with *Salmonella pullorum*. That the kind of ration has a marked effect on mortality from this disease has been reported by Roberts, Severens, and Card.[6]

In all the tests without sodium sulfamerazine in the feed the mortality among chicks inoculated with the treated culture was significantly greater than that among chicks inoculated with the untreated culture. This suggests a greater virulence of the treated culture. In all tests, except the first, when the treated culture had been grown in the 200-mg. concentration for only 36 days, the weights of the surviving chicks at 21 days were less for chicks inoculated with the treated than for those inoculated with the untreated culture whether or not sodium sulfamerazine was in the feed. This is also suggestive of a greater virulence of the treated culture. The average weight of all surviving chicks inoculated with the untreated culture is 128.8 gm. and 118.0 gm. for those inoculated with the treated culture. The difference is 10.8 gm. with a standard error of 2.94. The odds against this occurring by chance are more than 15,000 to 1.

The greater virulence of the treated culture in the later tests (tables 2 and 3) could be the result of a decreased virulence of the culture grown in normal medium without sodium sulfamerazine or to an increased virulence of the culture grown in the presence of sodium sulfamerazine. Whatever the explanation may be the results clearly indicate a greater virulence of the treated culture.

SUMMARY

Salmonella pullorum was grown in a medium containing sodium sulfamerazine. The medium with 200 mg. of sodium sulfamerazine per 100 ml. was markedly inhibitory to the growth of the untreated culture grown in normal medium. This could be interpreted as an acquisition of tolerance. However, the results indicate that the culture grown in the presence of sodium sulfamerazine for a long period of time was more virulent than the culture grown in the same kind of medium without sodium sulfamerazine when judged by mortality and weight of surviving chicks.

[5] Severens, J. M., Roberts, E., and Card, L. E. A study of the defense mechanism involved in hereditary resistance to pullorum disease of the domestic fowl. Jour. Infect. Dis. 75: 33–46. 1944.

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VIVIPAROUS GROWTH IN IMMATURE BARLEY KERNELS[1]

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INTRODUCTION

That a cell or group of cells will continue to grow and multiply so long as the environment is suitable seems axiomatic. The embryo of a barley kernel developing in a spike enlarges rapidly for a time; then growth slows down and practically ceases. The endosperm continues to enlarge for a considerably longer time, until growth and starch deposition cease; then water loss and shrinkage of the seed to an air-dried condition follow. The seed remains relatively inactive until placed in an environment favorable for germination, when the embryo absorbs water and oxygen and resumes growth. In an earlier report (*12*) [2] it was implied that an inadequate supply of water to the embryo was responsible for the failure of the embryo to continue growth uninterruptedly to the seedling stage while still attached to the parent plant. The experiments reported herein were conducted to test that implication by comparing such viviparous growth with the normal development of the barley seed.

MATERIAL AND METHODS

Two pure lines of cultivated barley—one a six-rowed variety (Manchuria, C. I. 2330 [3]; *Hordeum vulgare* L. emend. Lam.) and the other a two-rowed (Hannchen, C. I. 531, *H. distichon* L. emend. Lam.)—were grown in the greenhouse at the Plant Industry Station, Beltsville, Md., in the winter and spring of 1942. Flowers of each variety were emasculated and sib-selfed. A very good set of seed resulted in the Manchuria spikes, but because of poor pollen only a few Hannchen spikes were well filled. These were allowed to develop for 7 days and 9 days, respectively, when some of the spikes of each variety were treated to insure vivipary in some of their growing kernels by laying bare the embryo faces and providing them with a constant supply of water as previously described (*14*). The greenhouse temperature varied considerably, but a comparison with kernel growth obtained

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[2] Italic numbers in parentheses refer to Literature Cited, p. 309.

[3] C. I. indicates an accession number of Division of Cereal Crops and Diseases.

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under constant temperatures (*13*) indicated that the growing temperature averaged about 21° to 22° C. Samples of five or more Manchuria kernels from both the untreated and the treated spikes of the same age after pollination were taken daily. Hannchen kernels were sampled every third day. The samples were killed and fixed in Craf fluid, embedded in paraffin, and sectioned sagittally 15μ thick. For the most part, Heidenhain's iron alum haematoxylin and Delafield's haematoxylin with safranine were used as stains.

A series of 5 to 8 typical but selected median sections was magnified approximately 10 times with a Leica enlarger and projected upon a sheet of 5 by 7 Kodabromide glossy paper. When this was developed as a negative print, developmental changes from day to day could be followed easily and quickly.

Since the observations on the Hannchen variety simply corroborated those with the Manchuria variety they are not reported herein.

For studying the development of starch in the kernel, sib-selfed Manchuria kernels sampled in January and February 1940 were used. Since the greenhouse temperatures then were considerably lower than when the 1942 material was grown, growth in the former was slower; but by comparing embryo sizes a series of samples that corresponded closely to the samples taken daily in 1942 was selected. The 1940 samples were sectioned in the same way as the 1942 ones, and the progress of starch development was studied by staining sections progressively and very lightly with very weak iron alum haematoxylin and then treating them with iodine-potassium-iodide solution.

OBSERVATIONS

As previously reported (*12*, *14*,) vivipary can easily be induced in the growing barley kernel by supplying sufficient moisture to the face of its exposed embryo surface. Treatments given 6 to 9 days after pollination resulted in the appearance of seminal roots and the beginnings of plumule elongation on the fifteenth day after pollination. Probably because of differences in water absorption by different kernels, root and plumule emergence from the treated kernels were not simultaneous. The typical appearance of treated spikes was illustrated (*14*, *fig. 10*). Occasionally vivipary is induced in kernels wrapped in wet cotton without removing the lemma over the embryo. This indicates the availability of water as the chief factor in producing vivipary.

Studies of the development of the kernel of Manchuria barley at 20° C. have shown that 1 day after pollination the fertilized egg cell has divided once and the fusion nucleus has produced about 16 naked endosperm cells located at intervals in the undulating sheet of cytoplasm lining the egg sac (fig. 1, *A*). Four days after pollination (fig. 1, *B*) there are approximately 64 embryo cells and about 100,000 endosperm cells as estimated by extrapolation of the 20° curve (*13*, *fig. 2*). These endosperm cells form a continuous mass in the lower fifth of the embryo sac. Those adjacent to the embryo are the densest and most tightly packed. The remaining endosperm cells form a layer around the inside of the embryo sac, 2 or 3 cells thick on the chalazal side and only 1 cell thick on the opposite side. The distal ones are the last to get cell walls. By the fifth day after pollination (fig. 1,

C) the cavity within the embryo sac is filled with endosperm cells practically all of which have cell walls. The embryo is still undifferentiated on the sixth day after pollination (fig. 1, *D*). Meanwhile the ovule, which was about 1.6 mm. long on the first day after pollination, has grown to a length of about 5.4 mm.

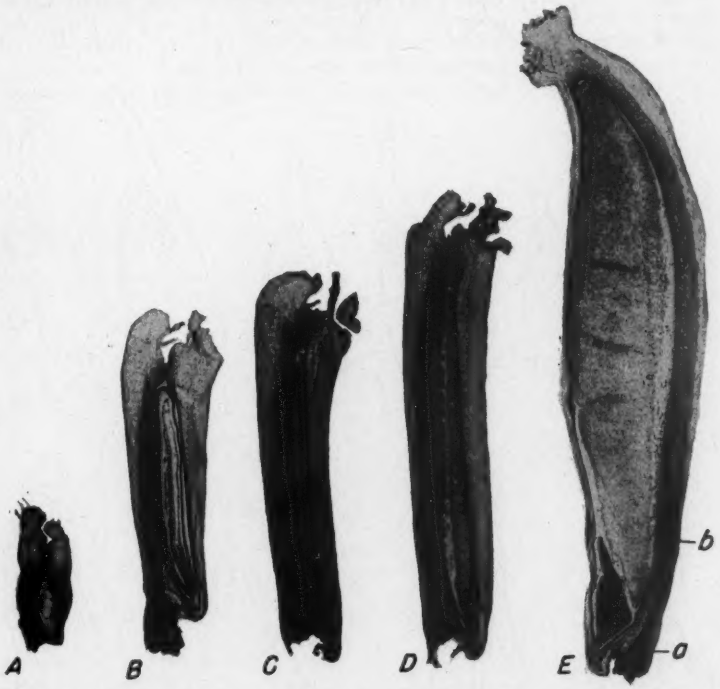


FIGURE 1.—Sagittal sections of barley ovules. *A*, One day after pollination, showing embryo sac containing antipodals, 2-celled embryo, and 6 of the 16 endosperm cells. *B*, Four days after pollination, when the endosperm cells extend full length of embryo sac and fill the lower fifth of it. *C*, Five days after pollination, when endosperm cells fill the embryo sac. *D*, Six days after pollination, when differentiation has just begun in the embryo. *E*, Eight days after pollination, showing fibrovascular bundle in the lower end of the furrow at the right; about 250 vessels are present at the level of the lower end of the embryo (*a*) and only about 25 at the level of the upper tip of the scutellum (*b*). All × 13.

On the day of treatment the Manchuria ovules have attained a length of approximately 7 mm. and the embryos an average length of 0.56 mm., which is equivalent to about 8 days' growth at 20° C. The plumule and primary root have begun to show differentiation; thereafter both treated and untreated kernels grew rapidly. The outlines of approximately median sagittal sections of embryos of Manchuria barley, beginning on the day of treatment for vivipary and at daily intervals thereafter, are shown in figure 2. The treated embryos

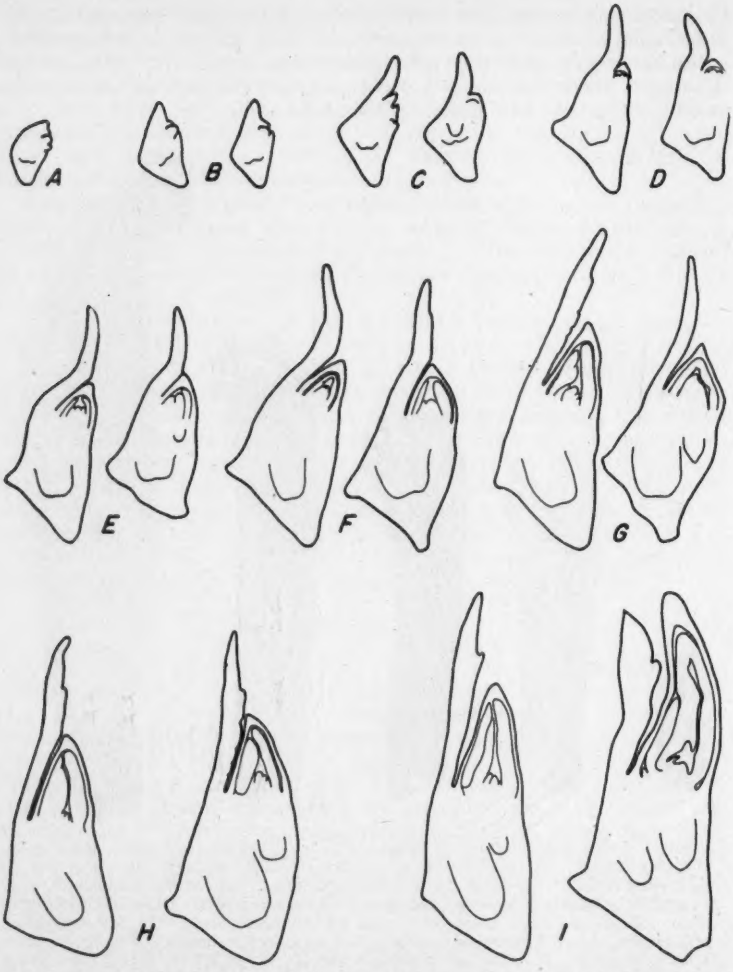


FIGURE 2.—Outlines, drawn with camera lucida, of median sagittal sections of barley embryos, 7 to 15 days after pollination. *A*, Seven days after pollination, the time of beginning of treatment to produce vivipary. *B* to *I*, Successive daily stages of embryos, untreated at the left and treated at the right; vivipary evident in *I* (right) 15 days after pollination. All × 20.

from 10- to 13-day samples are slightly smaller than those untreated, probably because of a slightly lower growing temperature brought about by evaporation of water from the treated spikes. The embryos in the untreated spikes apparently begin to lose moisture about 11 days after pollination, since the abscutellar surfaces of the embryos are noticeably less convex and turgid than those in the treated sam-

ples. As it is not certain that any of the figures show the exactly median section, the apparent internal differences are of little significance. The dimensions of the embryo outlines (fig. 2), however, correspond rather closely to the average size of embryos in approximately five kernels in each sample. These dimensions, as plotted in figure 3, are the total length of the embryo and the length of the growing axis. The latter is the distance between the extreme distal extension of any part of the plumule and the end of the coleorhiza, not including seminal roots. The total length of the normal, untreated embryo exceeds that of the treated from the eighth to the fifteenth day. The

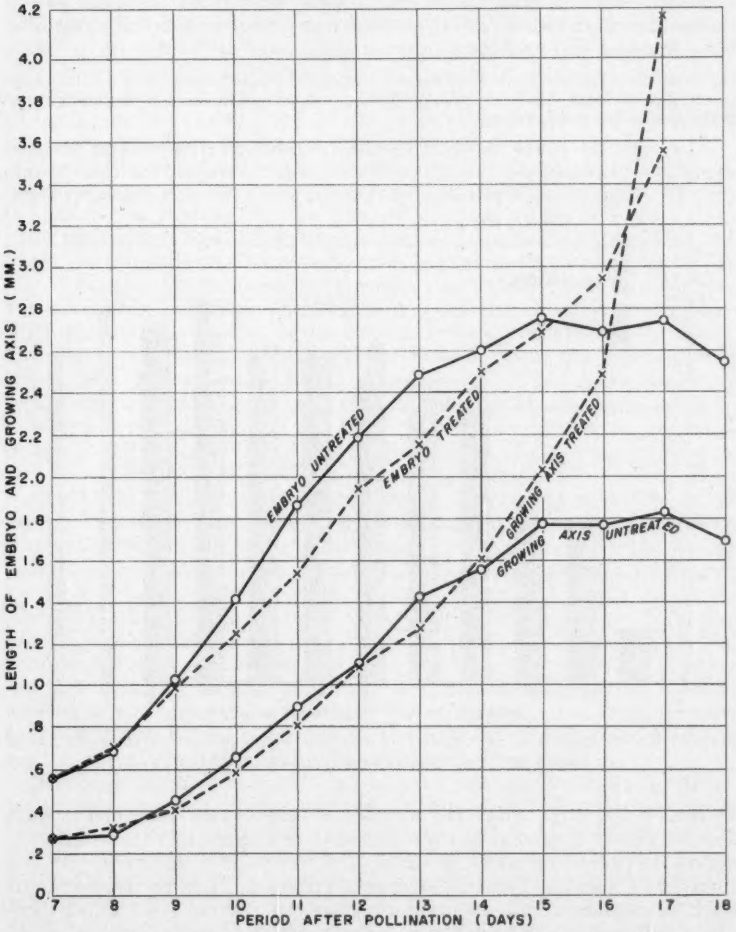
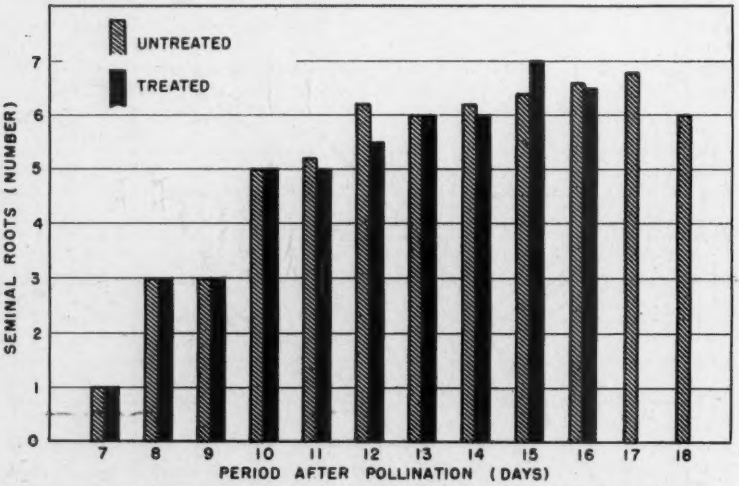


FIGURE 3.—Curves showing growth in length of embryo and of growing axis in untreated barley kernels and in those treated to produce vivipary.

curve of growth of the untreated embryo begins to be inflected on about the thirteenth day and flattens off sharply at 15 days. On the other hand, the growth of the treated embryo is rapid and continuous as shown by the uniform and steep slope of the growth curve. Similarly, the growing axis of the untreated check is longer than that of the treated embryo from the ninth to the thirteenth day; on the thirteenth day inflection begins in the untreated check and becomes complete on the fifteenth day after pollination. The treated embryo shows a very steady and rapid growth from the ninth to the seventeenth day, when the last samples were taken. The length of the growing axis overtakes that of the embryo on the sixteenth day. It is clearly evident from these curves that there is no dormant period in the development of embryos that have been successfully supplied with water. This conforms to the statement of Eyster (*3, p. 576*):

> So far as observations have been made, viviparous maize plants have a continuous development from the fertilized egg to the mature plant so that dormancy and germination are not involved.

The seminal roots in each kernel of both untreated and treated samples were counted "in the section" and averaged for the sample (fig. 4). The average number of seminal roots for this variety (Man-



—FIGURE 4.—Comparison of number of seminal roots in untreated embryos of barley and in those treated to produce vivipary.

churia) is 6.8 (*15*). Since the number of individuals examined in each sample rarely exceeded 5, no significant difference in root number is shown between normally growing and viviparous embryos. If the number of seminal roots is used as a criterion, embryo development may be considered at its maximum for the normal seed at 15 days after pollination. The normal embryo bears 5 to 11 seminal roots and root primordia, depending upon the variety (*15*).

It is interesting to note that an immature ovule after being harvested and dried will germinate immediately without either growing larger or becoming more fully differentiated. As shown previously (*15*), the seed may be considered viable whenever three seminal roots are demonstrable. From figure 2 it is seen that roots and plumule do not emerge from the treated embryo until it has reached the maximum development of the untreated embryo, namely, at 15 days after pollination at 20° C. The failure of the treated ovule to grow into a seedling at an earlier stage, as does the embryo of a seed harvested when immature, may possibly be explained by the presence, in the treated ovule, of the "embryo factor" suggested by Kent and Brink (*8*).

The normal-sized viviparous embryo extends its plumule and seminal roots until the nutrients available in the kernel are exhausted. Further growth can be induced by supplying a nutrient solution to the absorbent cotton wrapped around the treated spike. One such viviparous seedling continued to grow until the culm had attained an over-all length of more than 50 cm., when the invasion of the cotton by micro-organisms precluded further growth.

Normally, water is supplied to the growing kernel from the fibrovascular system of the culm by a branch bundle passing through the pedicel of the kernel. At the level of the base of the embryo 8 days after pollination the fibrovascular bundle of the pedicel supplying the floret contains about 250 vessels, each having a diameter of 5μ to 10μ (fig. 1, *E*, *a*). At the higher level where the mature kernel is attached, the number of vessels in the bundle is much less, because many have diverged into the bundles of the glumes, lemma, and palet of the floret On entering the kernel, the bundle divides to form the 2 lateral, the dorsal, and the ventral, or furrow, bundles (*7*). The furrow bundle is by far the largest of the 4, and about 25 vessels are distinguishable in it in transverse sections at the level of the upper end of the embryo of the 8-day kernel (fig. 1, *E*, *b*). Proceeding distally in the kernel, the vessels decrease in number until the last terminates at about nine-tenths of the distance to the distal end of the embryo sac. The number of vessels in the ovule appears to remain constant after about 8 days' growth. Since there are no vessels passing directly to the embryo, all water and dissolved nutrients must reach the embryo through adjacent tissue, which in turn is supplied with materials carried in the vascular system. The young embryo, then, is at the most favorable location for obtaining its supply of water and nutrients. It grows rapidly and at the end of 15 days has reached its maximum size and differentiation, and, in the normally growing ovule, without extending the plumule or radicle. The endosperm continues to grow thereafter and about one-third of the mature air-dry weight of the seed is added after the fifteenth day after pollination.

Although in the present study vivipary was produced readily when water was supplied to the exposed embryo area, naturally occurring vivipary is apparently rare. In 1909 or 1910 A. G. Johnson, of the Bureau of Plant Industry, Soils, and Agricultural Engineering, examined a sample from a meadow of green, standing timothy in southern Indiana, the ovules in which had produced seedlings in the spike after a protracted wet spell. J. H. Martin, of the same Bureau, has told the writer that this is a common occurrence in sorghums in the

southern Great Plains. Unlike the small, freely borne and easily dried-out spike of barley, or the maize ear adequately protected from external moisture by the husks, the dense sorghum head is exposed to drenching by rain and in protracted wet, humid periods the embryos may be supplied with enough moisture from the atmosphere to continue growth into seedlings. On the other hand, citations of the frequently observed "sprouting of grain in the shock" are not germane. These are merely instances of the germination of seeds that usually have matured on the stalk and dried down still further after harvest before absorbing enough rain water to produce germination.

Manifestly the lack only of water causes the embryo to go into the resting stage, since the only requirement for growth that was lacking in the untreated embryo, but available to the viviparous embryo, was distilled water. This is true not only for the spring-type variety Manchuria but also for three winter sorts that had proved to be extremely dormant when mature (*16*). If water is prevented from reaching the embryo at any stage after it has produced three seminal roots, a seed rather than a viviparous seedling is produced. In normal development the percentage of moisture in both embryo and endosperm decreases, slowly at first and then more rapidly, and the mature seed is the result.

In describing the progress of starch deposition in the barley kernel, Johannsen (*7*) stated that in two-rowed barley starch grains first appear within the endosperm cells when the kernel is 6 to 7 mm. long (about the stage when treatment to induce vivipary was given in the present study). He reported that starch first appears in the oldest endosperm cells, that is, those within the flanks of the upper part of the kernel but below the tip and that the younger peripheral region fills later. He believed that the endosperm cells can divide only until starch begins to be deposited in them.

In figures 5 and 6 are shown near-median sagittal sections of Manchuria barley kernels growing normally at 21°+ C. from the second to the fifteenth day after pollination. Development in them has progressed slightly further than in those of the same age grown at a constant temperature of 20° (fig. 1). Iodine-potassium-iodide solution stains mature starch grains deeply and younger grains less so or not at all. Very young, unstained starch granules may often be detected in the cells at higher magnifications.

Starch is clearly seen in the ovary wall at the distal end of the ovule on the second day after pollination (fig. 5, *A*). At higher magnifications starch grains progressively decreasing in size and age downward on the furrow (ventral) side, with a few very young grains scattered through the cells of the dorsal wall, were visible. By the third day (fig. 5, *B*), stainable grains appeared along the entire length of the furrow in the tissue surrounding the fibrovascular bundle and smaller, lightly staining grains were evident in the dorsal wall. On the fourth day (fig. 5, *C*), the dorsal wall had still more and larger starch grains and the furrow region had an abundance of starch. Up to the fourth day starch is apparent in maternal tissue only. The first appearance of starch in zygotic tissue occurred on the fifth day after pollination (fig. 5, *D*), when very small grains had appeared in the distal end of the endosperm mass, which by that time entirely filled the cavity of

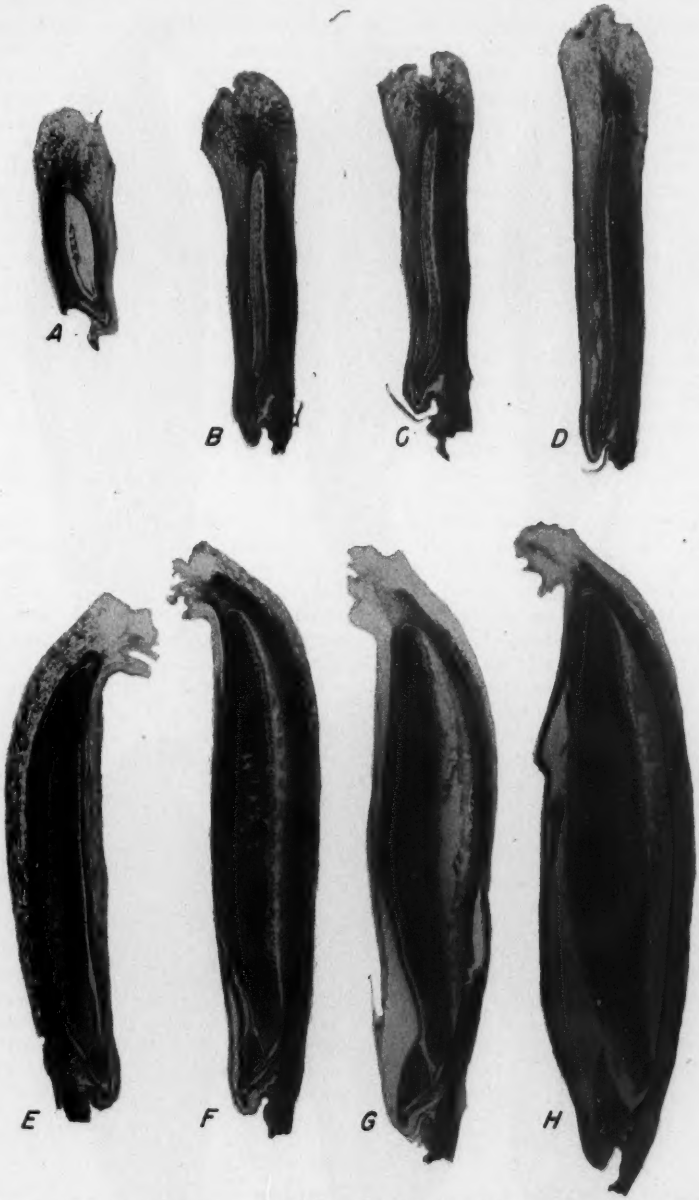


FIGURE 5.—*A* to *H*, Successive daily near-median sagittal sections of untreated barley kernels showing position and extent of starch deposit from the second to the ninth day after pollination (stained with iodine-potassium-iodide solution). × 13.

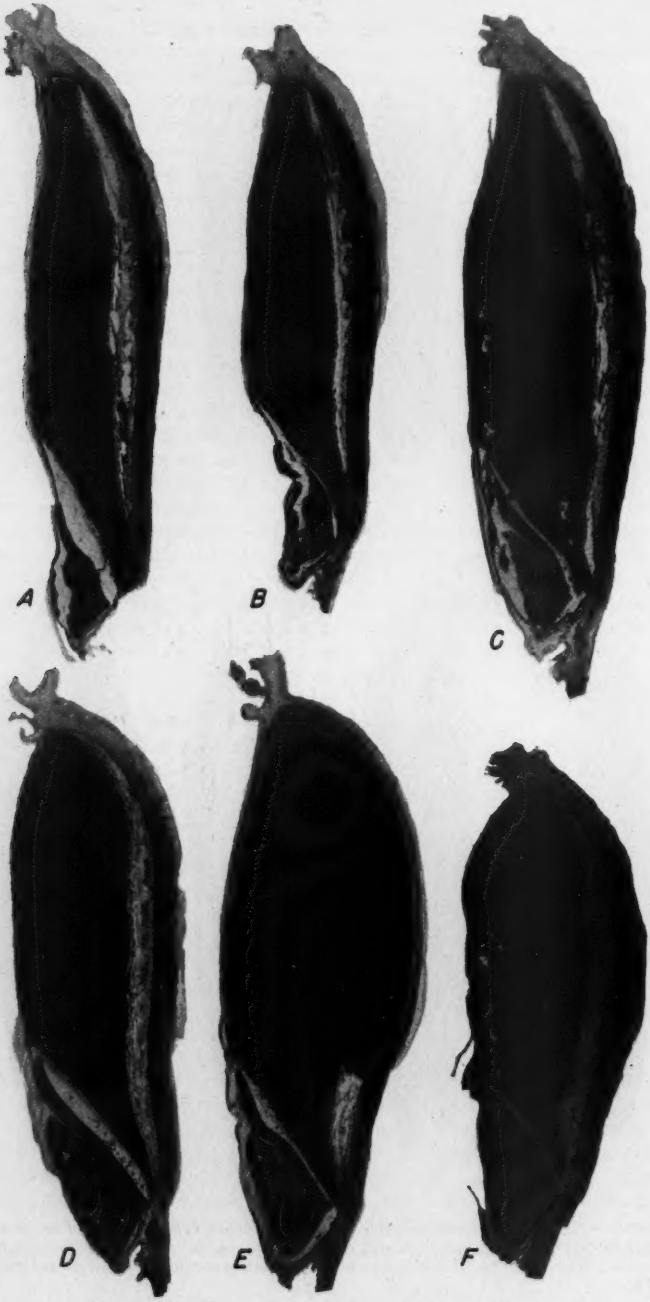


FIGURE 6.—For explanatory legend see opposite page.

the embryo sac. From that time on, endosperm starch increased rapidly in amount and density. On the sixth day (fig. 5, *E*) there were a few small grains in the basal third of the embryo. Starch was more abundant in the embryo on the seventh day (fig. 5, *F*) but was still confined to the lowest third; but by the eighth day it had appeared in the coleoptile and in the upper tip of the scutellum (fig. 5, *G*). In later stages (figs. 5, *H*, and 6), the ground tissue of the embryo was well supplied with stored starch, ideally located for ready utilization during germination. The formation of dense starch in the endosperm had not yet progressed downward to the level of the embryo until the eighth day (fig. 5, *G*), but by the ninth day (fig. 5, *H*) it had packed the cells to almost the proximal tip of the endosperm mass. The natural position of the embryo with the scutellum rather closely applied to the endosperm (fig. 6, *F*) was accidentally shifted in this and several other sections (fig. 6, *A*, *C–E*) during the killing, fixing, embedding, and sectioning of the kernel. In these near-median sections, the endosperm next to the embryo is thinner than it is in either flank, chiefly because of the keeled surface of the scutellum.

As has been stated, the endosperm cells had completely filled the cavity of the embryo sac and 5 days after pollination they were actively growing and multiplying. Two days later, when the treatment to induce vivipary was given, the moisture content of the ovule was approximately 80 percent (*6*) and the ovule had an oven-dry weight of about 2 mg. As shown in figures 5 and 6, the 8 days between treatment and appearance of vivipary is a period of extremely rapid growth and deposition of starch in the endosperm. This starch deposit is the main cause of increase in lateral and dorsiventral diameter and of decrease in moisture content up to the end of kernel growth. Fifteen days after pollination, when vivipary is evident in treated spikes, the dry weight of normally growing untreated ovules had increased to about 23 mg. while the water content had dropped to only about 60 percent. The air-dry weight of mature greenhouse seeds of Manchuria barley at 12 to 13 percent moisture is about 38 mg. per kernel. If a moisture content of 12 percent is assumed, the dry weight would be 33+ mg.

During early development the zygote lies in a lake of cell sap, which supplies it unstintedly with water and nutrients. The formation of endosperm cells under the scutellum tends to impede the passage of nutrients by osmosis from the furrow bundle, but enough still gets through to permit maximum growth of the ovule until inflection of the embryo growth curve on the thirteenth day after pollination. As kernel development progressed, the deposit of starch pushed the absorbing scutellum farther and farther away from the source of nutrient supply in the vascular bundle of the furrow. Only its lower end remained approximately in its former position. At the beginning of inflection of embryo growth on the thirteenth day (figs. 3 and 6,

EXPLANATORY LEGEND FOR FIGURE 6

A to *F*, Successive daily near-median sagittal sections of untreated barley kernels showing position and extent of starch deposit from the tenth to the fifteenth day after pollination (stained with iodine-potassium-iodide solution). × 13.

D), only this lower tip and the lower end of the ventral side of the embryo were readily accessible to the furrow, and these were being dehydrated. The furrow region was the last to remain green, and starch continued to be laid down in the young endosperm cells adjacent to it until desiccation of the kernel stopped all growth. This increasing interposition of gradually dehydrating starch-filled endosperm cells between the scutellum of the embryo and the source of supply of water and nutrients in the furrow bundle appears to be a sufficient cause for the inflection of the growth curve of the untreated embryo on the thirteenth day after pollination (fig. 3).

DISCUSSION

According to Gray (*5, p. 882*) a seed is "the ripened ovule, consisting of the embryo and its proper coats." Gager (*4, p. 453*) said that with few exceptions "the distinctive features of a seed is a resting embryo." The stage of maturity at which drying down may occur without sacrificing viability may vary considerably. Barley kernels taken from the spike as early as 9 or 10 days after pollination will, on drying, become "seeds" that are able to germinate and produce plants that later develop mature seed. Sections of embryos of these very young seeds show a very short plumule with the beginnings of about 3 leaves and the rudiments of 3 seminal roots (*15*). On the other hand, under good growing conditions embryos of barley ovules left to mature on the plant will attain a much greater size, with well-developed plumules and the normal complement of seminal roots, which may total as many as 10 or 11 in individual seeds of certain varieties.[4] Recently Kent and Brink (*8*), in growing excised immature barley embryos on sterile media, found that, while such embryos normally germinate within 2 or 3 days and become very small, spindling seedlings, the addition of certain substances, such as water extracts of dates and bananas, milk, and tomato juice, will delay germination and allow the embryos to grow and develop *as embryos* until they reach or surpass the size of embryos matured on the plant. Such an "embryo factor," present in normally growing barley kernels but absent in excised immature kernels, should account for the absence of further pregermination development in the embryos of immature barley seeds.

The embryo of the untreated normally maturing seed loses water. The insufficient water supply precludes starch digestion and nutrient transfer, and the embryo goes into the resting condition. A resting stage may be forced upon an immature embryo by drying, or the seed may attain "maturity" through natural desiccation. On the other hand, if water is supplied constantly to the developing embryo, the embryo not only attains the maximum size and development of that in the normally matured seed in about 14 days from pollination but it continues to grow thereafter and form the seedling, with no inflection of the growth curve. In the viviparous seedling the conditions of youth are largely maintained; there are ample nutrients available in the endosperm adjacent to the scutellum, starch-digesting enzymes

[4] In 1943 when barley seeds were germinated in petri dishes 1 seed of the variety Alpha produced 11 seminal roots and 1 of the variety Primus 10.

are present in the secretory layer of the scutellum, and sufficient water reaches all parts of the embryo to provide for uninterrupted growth. Such a seedling, if pricked off, is able to produce a plant that yields normal seed.

In a viviparous seedling, the course of normal growth as occurring in juvenile plants or plant parts obtains and growth proceeds upon the compound-interest principle. The cell walls are soft and permeable, and there is no mechanical limitation to cell division. Successive increments are added to the "principle" of reactive material, but, as has been noted (*11*), growth rate is conditioned by nutritional opportunities. As strengthening, supporting, and nutrient materials are laid down in the growing entity, nutrition is impeded by desiccation, cell growth and division are retarded, and the grand period of growth suffers its inflection. Carried still further, these aging factors result in dormancy or death.

The conception of vivipary herein presented gets considerable support from the findings of Eyster (*3*), who stressed the fact that vivipary is a fundamental and primitive character and cited its occurrence in all great divisions of the plant kingdom (*3*). He attributed the dormancy of the embryo of the mature seed to insufficient water supply and stated that if developing maize ears are kept under suitable conditions of moisture the sporophytes appear to have a continuous growth from the fertilized egg to the new plant (*1*). Moreover, as in normal immature barley ovules, he found that viviparous kernels of maize that had been forced into dormancy by artificial drying germinated when they were exposed to suitable growth conditions (*3*).

A large proportion of the viviparous kernels found in maize possess "deficient" endosperms. Eyster (*2, p. 250*) stated:

> . . . practically all of the kernels with reduced endosperm have viviparous embryos due to the very close linkage between re_2 vp_2.

Naturally, a reduced or deficient endosperm would tend to make less effective the starch endosperm block to the passage of water from the fibrovascular system to the embryo. This would facilitate vivipary.

Mangelsdorf (*10, p. 493*), however, attributed the phenomenon of vivipary to the action of genes, citing "fifteen different genetic factors and nine distinct characters" involved in its inheritance. He said (*10, p. 490*):

> Dormancy of the seed during development is so general that it may almost be taken for granted. Yet we find that at least fifteen different genetic factors are involved in maintaining dormancy and we can scarcely suppose that this is more than a sample of the total number.

He (*9*) used the stage of kernel development at which vivipary occurs as a basis for classifying his different types of the phenomenon. On the other hand, Eyster (*3*) found different stages of vivipary in the same ear of maize. Similarly, it was found in the present study that kernels on the same barley spike showed different stages of root and plumule emergence.

To the writer, Mangelsdorf's interpretation seems a greatly exaggerated complexification of a very simple phenomenon. Vivipary is the *natural* course, and probably there are many ways whereby the normal desiccation of the growing embryo may be prevented, any one

of which will allow vivipary. It seems probable that the so-called vivipary genes reported by Mangelsdorf (*10*) function by varying the structure of the kernel; this variation in turn may restrict the quantity of water available to the growing embryo.

If, as Eyster (*3*) believed, the typical resting condition of seeds is an adaptation forced on the organism to insure survival, vivipary should occasion no surprise. The phenomenon that demands explanation is the *dormancy* of the mature seed. To the writer it seems clear that the maturation of the seed is brought about by structural factors that inhibit growth of the embryo by gradually cutting off its supply of water. In barley, the most likely block to water passage consists of the starch-packed endosperm cells massed between the embryo and the fibrovascular bundles lying in the still chlorophyllous tissue of the furrow.

SUMMARY AND CONCLUSIONS

Viable resting embryos may be induced in barley by either drought or harvest at any time after their earliest viability stage when three seminal roots are present. Their development into seedlings is proof that such seeds have sufficient stored nutrients except water and oxygen.

Under optimum natural growing conditions the embryo becomes dormant after reaching the maximum size and development that is possible under the conditions of gradual desiccation in the maturing seed.

When distilled water is supplied to the abscutellar surface of the growing embryo, growth is continuous and vivipary occurs.

Ovules treated on the seventh day after pollination to induce vivipary developed similarly to normally growing ones in all observed features up to the thirteenth day, except that after the ninth day the embryo appeared to be more plump or turgid in the treated ovule than in the untreated one. Growth of the plumule was first visible in the viviparous ovule on the fourteenth day. Seminal roots had emerged and the plumule was actively growing on the fifteenth day after pollination.

There was no significant difference in the number of seminal roots on the viviparous and the normally matured embryos.

In a normally growing ovule a resting stage is forced upon the growing embryo, while in the ovule treated to induce vivipary no such interruption to growth, or even lessened growth rate, occurs.

In the normally growing ovule water adequate for vivipary is blocked off probably by the interposition of a dense layer of starch-bearing endosperm cells between the embryo and the fibrovascular bundle in the furrow.

The thesis is submitted that vivipary, rather than itself being a genetic character, is merely the result of any condition, character, or seed deficiency that will allow the access of water to the embryo.

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TIP BLIGHT OF GARDEN PEA[1]

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INTRODUCTION

In 1941, plants of garden pea (*Pisum sativum* L.) with dead tips were noted in a seed-treatment experiment near Madison, Wis. Plating of diseased tissue on agar yielded a species of *Pythium* which upon inoculation of potted plants in the greenhouse produced similar symptoms. Correspondence with Dr. W. T. Schroeder, New York Agricultural Experiment Station, revealed that he had noticed the disease in the same locality in 1940 and had also isolated *Pythium*, although no controlled inoculations were made. The disease was seen in commercial fields of canning peas in 1942 and 1943. Damage was generally distributed, but slight, with the exception of one field in 1942 where 40 percent of the plants had killed tips.

In 1946, in a nursery near Madison, an average of 6 percent of the plants and 10 to 15 percent of some lots had killed tips. This amounted to considerable damage since most of the lots were single-plant progenies planted for further selection. The disease was observed in several commercial fields during that season, one of which had 20 percent of the plants diseased in a 2-acre section of the field. Apparently the same species of *Pythium* was isolated in all cases from diseased plants and it was tentatively identified as *Pythium ultimum* Trow. Because of the unusual nature of the disease a more detailed investigation was made, a preliminary report of which was published in 1947 (*4*).[2]

Pythium ultimum was described by Trow (*13*) in 1901 as a saprophyte. Since that time it has been reported many times as a parasite. Middleton in 1943 (*8*) listed 148 plants as hosts and noted that it was perhaps the most common member of the genus in the United States and that it was found frequently in other countries. It has been reported frequently in association with damping-off and with root rot of peas (*1*, *10*, *11*). No record has been found of this, or any other species of *Pythium*, occurring on the above-ground foliage of the pea plant independently of infection originating below the soil level.

Middleton (*7*) in 1941 described a rot of roots, crowns, buds, and petioles of rhubarb incited by *Pythium anandrum* Drechsler, *P. oligandrum* Drechsler, and *P. ultimum*. Middleton et al. (*9*), in 1942 de-

[1] Received for publication March 29, 1948.

[2] Numbers in parentheses refer to Literature Cited, p. 324.

scribed a stem and lower-leaf rot of fibrous-rooted begonia incited by *P. debaryanum* Hesse, *P. splendens* Braun, and *P. ultimum*. *P. ultimum* was the most pathogenic on rhubarb and the most frequent on begonia. In both cases rot of the aerial plant parts followed infection of the lower parts. Tompkins et al. (*12*) in 1939 found *P. ultimum* inciting a soft rot of the fruits of Zucchini and Mammoth Summer Crookneck pumpkin and of watermelon. Fruits of tomato, bean, eggplant, and field pumpkin became infected when inoculated without wounding and several other types of fruit tested became infected when inoculated through wounds.

Similar diseases of fruits, storage organs, and foliage parts (infected by progression of the pathogen from tissue at the soil level or below) have been reported as incited by still other species of *Pythium*. Drechsler (*2*) in 1925 found cabbage heads rotted by a *P. debaryanum* type with the fungus spreading out from the fleshy core and leaf bases to the lamella. A similar rot was produced when inoculation was made with several other species of *Pythium;* some species were not pathogenic. Harter and Zaumeyer (*5*) in 1931 reported a bean wilt, due to *P. aphanidermatum* (Edson) Fitz. (*P. butleri* Subr.), which started at the ground line and progressed up the lower stem to the lower branches. Wound inoculation at the cotyledonary node resulted in infection.

Elliott (*3*) in 1943 described a stalk rot of the lower nodes of corn incited by *P. aphanidermatum* (*P. butleri*) and reproduced the disease in the field and greenhouse. The crown and roots were not involved when the disease was encountered in nature and the roots were not infected when inoculated in the greenhouse. The activity of the fungus in the stalks ceased abruptly and demarcation of invaded tissues was strong. Wallace (*14*) in 1944 reported a species of *Pythium* or *Phytophthora* which incited damping-off and foot rot of papaw in East Africa but stated that most infections occurred through leaf and fruit scars on the upper part of the plants, spreading to stems, leaves, and fruits. These two are the only reports found of independent infection of aerial parts of plants, other than fruits or storage organs, by species of *Pythium* and in one of them the identity of the fungus is uncertain.

EXPERIMENTAL RESULTS

SYMPTOMS

Infection takes place in the bud or the axil of a young leaf. In the field, affected tissues are at first water-soaked with a fading of the green color. This stage does not last long, for invaded tissues are killed very rapidly. As desiccation ensues the tissue shrivels and the color changes to straw color to black (fig. 1, *A*) depending on conditions during drying. The fungus advances downward from the tip, rarely killing more than three to five internodes. Girdling takes place from the leaf axil with collapse of that node and consequent killing of the tip above it (fig. 2). Occasionally, girdling is incomplete with the result that a part or all of the leaf is killed and the tip of the plant survives. In exceptional cases one or more leaflets may be partially or entirely killed as a result of local infection rather than from that originating in a bud or leaf axil, while sometimes the entire plant, particularly if it is quite young, may be killed.

FIGURE 1.—Pea plants from nursery near Madison, Wis., showing effects of tip blight due to *Pythium ultimum*: *A*, Growing point and two nodes below it killed; *B*, later stage with lateral shoots started below blighted tip.

When a tip or leaf of a plant is killed, progress of the lesion halts abruptly and the line of demarcation at the junction of healthy and diseased tissue is sharp. The fungus is not visible macroscopically on the exterior of diseased parts, but often a cottony web of mycelium containing fruiting bodies appears in the hollow of the stems. Diseased plants have been found ranging in maturity from those just emerged to those in blossom. However, in two adjacent lots differing in size, a greater percentage of infected plants is generally found in the shorter plants.

As soon as the tip of a plant is killed new shoots are put out in the axils of lower leaves (fig. 1, *B*). If the plant is young, one shoot quickly gains ascendency over the others and the affected plant may be overlooked later in the season unless the later maturity is noted. On larger plants there is more competition among the new shoots.

FIGURE 2.—Pea plant from nursery near Madison, Wis., infected in leaf axil by *Pythium ultimum*. Top is beginning to wilt and lateral shoots have started.

All of them may grow for some time and as many as five of the upper ones may continue to be about equal in vigor.

Symptoms identical with those found in the field were produced by suitable artificial inoculation of young potted plants in the greenhouse (fig. 3, *A*). The water-soaked appearance of the tissues was evident within 48 hours and subsequent drying and discoloration were very rapid. When left on the greenhouse bench after the original treatment, the plants put forth new shoots from the axils of lower leaves. A type of symptom not seen in the field was produced in the greenhouse by spraying the entire plant with a suspension of mycelial fragments of the fungus. With 6 to 24 hours in a moist chamber, there were scattered over the leaves and stems many small water-soaked spots that dried to a very light brownish tint. If left in the moist chamber for 48 hours all plants were completely killed. In bud inoculations, when the tip escaped killing, one or more leaves or parts of leaves were frequently killed.

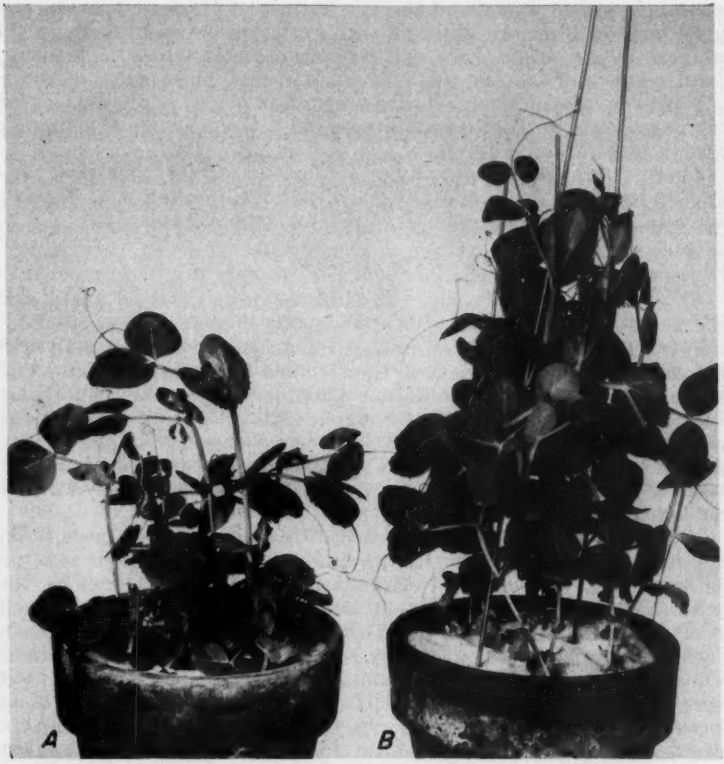

FIGURE 3.—*A*, Delwiche Commando pea plants inoculated in the terminal bud with *Pythium ultimum* and kept in moist chamber for 48 hours; note lateral shoots at bases of plants. *B*, Uninoculated.

THE CAUSAL ORGANISM

Isolations made from diseased plants when the disease was first noted in 1941 consistently yielded a fungus identified as a member of the genus *Pythium*. All isolations were from plants from the same plot. No effort was made to determine the species. In 1946, numerous isolations were made from diseased plants in the nursery at Madison and from material collected in two widely separated commercial plantings in Wisconsin. These consistently gave the same species and it was apparently similar to the one isolated in 1941, although no cultures made in 1941 were available for comparison. Collections from two commercial plantings in 1947 gave the same species upon isolation.

One of the isolates made from a plant in the Madison nursery in 1946 was selected as typical and a detailed examination was made. It was fast-growing, producing an abundance of white, cottony mycelium on potato-dextrose agar, and numerous oospores and

sporangia on the surface of the agar within 10 days to 2 weeks. It grew well on a variety of media with variation between media in the amount of aerial mycelium and fruiting bodies produced. Fruiting, both sexual and asexual, was best when a small block was cut from a vigorous culture on potato-dextrose agar and placed in distilled water. Germination of oospores was not observed. Germination of sporangia was by germ tubes only.

Spore sizes, sexual apparatus, germination of the sporangia, and growth habits of the fungus all agreed with the description of *Pythium ultimum* Trow. (*13*) as given in Middleton's monograph (*8*).[3]

PATHOGENICITY

When a species of *Pythium* was isolated from diseased pea plants in 1941 young pea plants of the Wisconsin Perfection variety about 5 inches tall were inoculated in the greenhouse by placing small agar blocks containing the fungus in the terminal buds of some plants and in the upper leaf axils of others. After inoculation the plants were kept in a moist chamber for 48 hours. All of the tips of the plants were killed regardless of the point of inoculation, and symptoms were similar to those seen in the field. The fungus was reisolated from some of the diseased plants. An equal number of uninoculated plants remained healthy.

Five isolates from material from the Madison nursery were used in preliminary inoculations in the greenhouse in 1946. There were no differences in type or degree of symptoms produced. Therefore, the isolate selected for detailed examination in the laboratory was used in all subsequent inoculations. Delwiche Commando and Wisconsin Perfection varieties of peas were used in the inoculation experiments. Since both varieties were very similar in their development and both were identical in reaction to the fungus, the variety has not been specified in reporting individual experiments. Except in temperature experiments, plants averaged 4 to 6 inches tall with four to seven leaves each when inoculated. All plants were grown in sterilized quartz sand in 6-inch clay pots and were watered on alternate days with a balanced nutrient solution.

Inoculation was tried first by placing agar blocks containing the fungus in buds of plants and in axils of young leaves. Considering only those left in the moist chamber for 48 hours, the tips were killed on each of 12 plants inoculated in the bud and on 15 of 16 inoculated in leaf axils. The fungus was recovered from all plants. Forty plants left uninoculated remained healthy. A second method of inoculation was to spray the entire plants with a suspension of mycelial fragments prepared by growing the fungus for 6 days on 100 cc. of a modification of Czapek's solution [4] in 250 cc. Erlenmeyer flasks at 28° C. and macerating the mycelium 30 seconds in a Waring Blendor. Of those left in the moist chamber 48 hours 17 of 18 plants were collapsed and dead 2 days after removal. The eighteenth plant

[3] Appreciation is expressed to Dr. John T. Middleton, Citrus Experiment Station, Riverside, Calif., who kindly checked the culture sent to him and confirmed the identification.

[4] KNO_3, 3 gm.; KH_2PO_4, 1.0 gm.; $MgSO_4.7H_2O$, 0.5 gm.; KCl, 0.5 gm.; $FeSO_4$, trace; dextrose, 30 gm.; water, 1,000 ml.

had two leaves alive at that time. No attempts were made to recover the fungus. None of the controls were diseased. A third means of inoculation was to place a drop of the suspension described above in the bud of the plant or in a selected leaf axil with a medicine dropper. In a representative experiment, of those left in the moist chamber for 48 hours, the tips were killed on 71 of 74 plants inoculated in the bud and on 29 of 66 plants inoculated in leaf axils. Twenty-three of the plants without the tip killed had part or all of the leaf killed at the node of inoculation. Isolations were made from 20 diseased plants of each type of inoculation and the fungus was recovered from each of them. Uninoculated plants in eight pots, averaging seven to nine plants per pot, kept in the moist chamber 48 hours, remained healthy.

Of the inoculations described only the plants that were left in the moist chamber 48 hours are itemized. Successful inoculations were secured on many more plants in these particular series of inoculations and in other series not discussed, although few other attempts were made to reisolate the fungus. Thus it was demonstrated that the fungus can incite the disease seen in the field.

After the pathogen was identified as *Pythium ultimum*, a common soil organism frequently associated with damping-off of peas and other crops, it became of interest to examine its potentialities in damping-off of peas in the greenhouse when mixed with the soil in pure culture. Flats of unsterilized greenhouse compost soil were prepared and 300 cc. of the mycelial-fragment suspension was thoroughly mixed in alternate flats. All flats were left at 24° C. for 7 days and the soil was kept moderately moist. Six rows of 20 seeds each of Delwiche Commando peas were planted in each flat and 1 inoculated and 1 check flat were placed at each of several temperatures. The results at 28° C. 19 days after planting may be seen in figure 4. There were 111 seedlings in the check flat or an average of 18.5 per row, an average emergence of 92.5 percent healthy plants. There were nine seedlings in the inoculated flat not showing advanced stages of damping-off, or an average emergence of 7.5 percent healthy plants.

Since the pathogen concerned was found to be an active damping-off agent of peas and there are other species of *Pythium* that act likewise, the question arose as to whether other species of the genus could cause the same type of above-ground disease. Another *Pythium* species, tentatively identified as *P. debaryanum*, was obtained from M. P. Backus, University of Wisconsin. It was compared with *P. ultimum* in a series of inoculations replicated twice at temperatures of 16°, 20°, 24°, and 28° C. and periods in the moist chamber of 24, 48, and 72 hours at each temperature. Inoculation of both was by drops of mycelial-fragment suspension, prepared as described above, in buds of the plants. The symptoms produced on the plants by *P. debaryanum* were the same in type as those produced by *P. ultimum* but were much less severe under all conditions tried. A typical response may be seen in figure 5, made 10 days after inoculation at 28° with 48 hours in the moist chamber. Here, where 100 percent of the plants had two to three nodes killed by *P. ultimum*, only 60 percent of the tips were killed by *P. debaryanum* and then only the uppermost one, or rarely

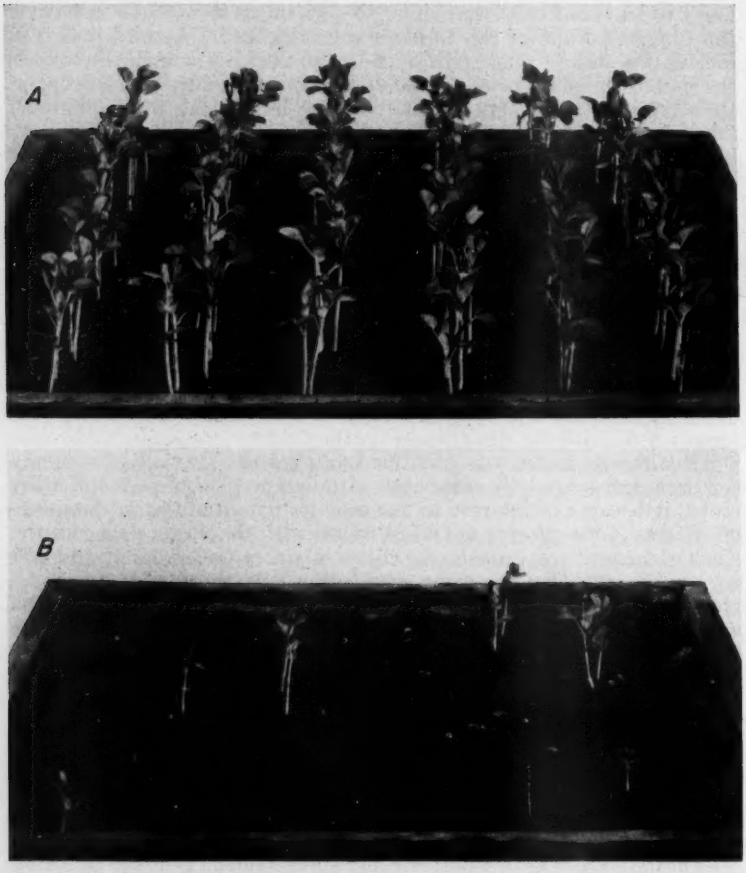

FIGURE 4.—Delwiche Commando peas 19 days after planting, 20 seeds per row, in greenhouse compost at 28° C.: *A*, Uninoculated; *B*, soil inoculated with *Pythium ultimum*.

two, nodes. No attempt was made to increase the virulence of *P. debaryanum* by repeated passages through the host.

EFFECT OF MOISTURE AND TEMPERATURE ON INFECTION

When inoculations were made by placing agar blocks in buds, percentage of infection and severity of the disease were increased by putting the plants in a moist chamber. However, over 50 percent of the tips of plants treated in this manner were killed with no exposure in the moist chamber. No infection resulted when agar blocks were placed in leaf axils unless the plants were left in the moist chamber at least 6 hours. When plants were sprayed with the mycelial-fragment

FIGURE 5.—Delwiche Commando peas grown in sterilized quartz sand at 28° C.: *A*, Uninoculated; *B*, inoculated in terminal bud with *Pythium debaryanum*; *C*, inoculated in terminal bud with *Pythium ultimum*. Plants placed in moist chamber for 48 hours after inoculation.

suspension, small spots or edges of the leaf tissue were killed with no moist-chamber treatment. These methods were not considered representative of what might be expected to happen in the field.

The inoculation treatment in which drops of mycelial-fragment suspension were placed in buds or leaf axils probably most nearly approached infection under field conditions. When this method was used, a period of 24 to 48 hours in the moist chamber was necessary for infection to take place. At higher temperatures (24° and 28° C.) the time required was shorter, as shown in table 1. Usually most of the tips were killed at these temperatures with an exposure of 24 hours, whereas at 16° it took over 72 hours in the moist chamber after inoculation to approach this level of disease. In all cases the percentage of infection was higher and the disease was more severe when the drops were placed in the bud.

TABLE 1.—*Effect of length and temperature of the inoculation period on tip blight following inoculation in the bud with Pythium ultimum*

Hours in moist chamber	Percentage of plants with tips killed at—			
	16° C.	20° C.	24° C.	28° C.
24	0	11.6	93.3	100.0
48	6.2	80.8	100.0	100.0
72	77.0	93.3	100.0	100.0

It was noted in the field and in the greenhouse that the progress of the fungus stopped as soon as the plants were in relatively dry air. Apparently only that tissue invaded when a high relative humidity prevailed was involved. Plants staked in the field after the initial observation rarely showed any extension of lesions and those left on

the greenhouse bench after tips were killed remained healthy below the original lesion. Six pots of such plants, averaging eight plants per pot, were replaced in the moist chamber 3 weeks after inoculation. They were removed at 2-day intervals. There was some further killing of stems and leaves of 80 to 90 percent of the plants removed after 2 days and 100 percent killing of the entire plant of those left 6 days.

Temperature had a marked effect on infection and development of the disease. Experiments were conducted in constant-temperature houses held at 16°, 20°, 24°, and 28° C. Moist chambers in which plants were placed after inoculation were in each of the houses. There was a reduction in temperature in the moist chambers due to the cooling effect of evaporation, temperatures there being about 13°, 17°, 21°, and 25°, respectively. Since the effect of the reduction in temperature during the inoculation period compared to that of the post-inoculation period was not determined, results are given on the basis of the temperatures of the latter period.

In all cases there was an increase in percentage of infection or severity of disease or in both from 16° to 24° C., with very little or no differences between 24° and 28°. The effects of temperature and length of time in the moist chamber were interrelated, for with a longer time in the moist chamber the percentage of infected plants at the lower temperatures increased. However, if plants at all temperatures were left long enough for infection to approach 100 percent at 16°, the disease involved more nodes as the temperature increased.

To determine the effect of pretreatment temperature on the reaction of the plants to the disease, plants grown at 16°, 20°, 24°, and 28° C. were distributed at each of the four temperatures immediately prior to inoculation. The plants were 18 days old and varied in size from 2 inches high with four nodes at 16° to 6 inches high with seven nodes at 28°. There were 5 pots with 8 to 10 plants each in each treatment. Two were left as checks and three were inoculated. Inoculations were made by placing a drop of mycelial-fragment suspension in the bud of each plant and the plants were left in the moist chambers 68 hours. Ten days after inoculation plants were rated in four classes as follows: (1) Tips dead; (2) one or more leaves completely killed; (3) no leaf entirely killed but lesions on leaflets or petioles; (4) healthy. A disease index was calculated for the plants in each pot [5] and the indices for the three pots in each treatment averaged. The results may be seen in table 2. The influence of temperature after inoculation was highly significant and there was a definite increase in disease index from 16° to 24°. The length of time in the moist chamber may have obscured some of the differences at the higher temperatures. The influence of preinoculation temperature was significant at the 5-percent level with a higher disease index at 20° than at 16° and 24°. It is doubtful if this relationship would be consistent in further trials.

[5] Number of plants in classes 1, 2, 3, and 4 multiplied by 3, 2, 1, and 0, respectively; total divided by three times the total number of plants, and result multiplied by 100.

TABLE 2.—*Effect of temperature before and after inoculation on pea plants inoculated in the bud with a drop of mycelial-fragment suspension of Pythium ultimum*

[Averages of disease indices from 3 pots of 8 to 10 plants each in each treatment]

Preinoculation temperature (° C.)	Disease index at temperature after inoculation indicated				Average [1]
	16° C.	20° C.	24° C.	28° C.	
16	51.1	88.0	95.5	96.3	82.7
20	61.1	94.4	100.0	97.8	88.4
24	48.6	86.2	93.0	90.0	79.5
28	48.7	94.3	98.0	97.8	84.7
Average [2]	52.4	90.7	96.6	95.5	

[1] Differences significant.
[2] Differences highly significant.

MSD at 5-percent level: 5.4.

Seeds were planted in inoculated and uninoculated soil in flats. After planting, one flat of each was placed at 16°, 20°, 24°, and 28° C. Stand counts were made 26 days after planting and the stand in each of the six rows of each flat averaged. The results are shown in table 3. The percentage reduction in stand rose from 52.9 at 16° to 91.9 at 28°.

TABLE 3.—*Effect of temperature on stand of peas in uninoculated greenhouse compost in flats and in the same soil inoculated with Pythium ultimum*

[Average stand per row from 20 seeds in each of 6 rows per flat]

Treatment	Stand at temperature indicated				Average
	16° C.	20° C.	24° C.	28° C.	
Uninoculated	15.3	18.3	15.6	18.5	16.9
Inoculated	7.2	5.5	3.5	1.5	4.4
Percent stand reduction	52.9	70.0	77.6	91.9	74.0

Petri dishes containing 20 cc. of potato-dextrose agar were inoculated with small blocks of agar cut from the edge of a rapidly-growing culture of *Pythium ultimum*. Four plates each were placed at constant temperatures ranging from 4° to 36° C. at 4° intervals. After 24 hours diameters of the colonies were measured at right angles. This experiment was repeated 3 times, and the average diameter of the colonies in the 12 plates at each temperature are shown in figure 6. The average disease index after inoculation and percentage reduction in stand are presented for comparison. Best growth of the fungus in culture was at 28° with a slight reduction at 32° and 24°. The stand-reduction curve parallels the growth of the fungus in culture. The disease index differs in that there is a sharper rise from 16° to 20°, less from 20° to 24°, and no differences between 24° and 28°. However, the trend is the same with greater pathogenic activity of the fungus at temperatures near the optimum for its growth.

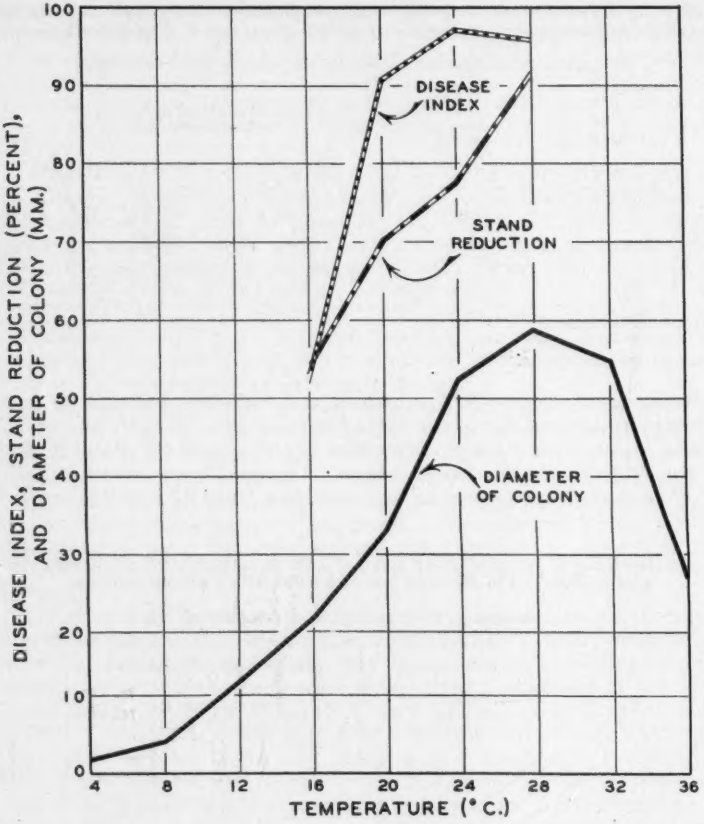

FIGURE 6.—Effect of temperature on diameter of colony of *Pythium ultimum* on potato-dextrose agar after 24 hours, on reduction of stand of peas in inoculated soil, and on disease index of bud-inoculated plants.

DISCUSSION

The effects of temperature on the foliage disease and on the damping-off phase in the range of temperatures tried indicate that increased severity at 24° to 28° is due principally to the effect of temperature on the fungus. There was a significant effect at the 5-percent level of preinoculation temperatures of the plants. The differences were small and the work would have to be repeated several times for the results to be accepted as conclusive.

In the field, the disease has been seen only after prolonged periods of cloudy and wet weather with at least one heavy rain in the period. Plants infected have ranged in maturity from those just emerged to those just beginning to blossom, but none have been seen infected after the blossom stage. This agrees with conditions for infection as

determined in the greenhouse. At average temperatures that would be expected during the early part of the season (16° to 20° C.) at least 48 hours of high humidity would be necessary for infection.

There is the question of how the inoculum gains access to the buds and leaf axils of the plant in the field. The fungus is common in the soil and has been reported frequently as an agent in damping-off of peas (*1*, *10*). No fruiting bodies of any kind have been found on external parts of lesions; thus spread from plant to plant probably is negligible. Possibly sporangia or oospores are splashed from the soil surface and those lodging in water drops on the plants originate the disease if suitable moisture conditions prevail long enough. Since pea foliage is waxy, collection of water drops would be expected more frequently in buds or leaf axils. McLaughlin (*6*) isolated *Pythium* (species undetermined) from the soil surface in all seasons of the year with a higher percentage of total isolations belonging to *Pythium* in cooler months. The lowest percentage was under conditions of high temperature and low moisture.

Damage to the crop consists in delaying the maturity of affected plants beyond that of the surrounding ones. The entire plant is rarely killed. The pods produced on delayed branches are usually too late to be of any value when the crop is harvested for canning. Although local damage has been severe in some cases, widespread losses have not occurred since the disease has been under observation. Because of the conditions necessary for disease development and the fact that the fungus has undoubtedly been present for a long time without causing great damage, it is not expected that the disease will become a serious factor in production of the canning crop.

In the nursery in 1946 there were present a large number of breeding strains and varieties representing a wide range of types of canning peas. When the disease appeared the opportunity was taken to examine the various lots in detail for evidences of varietal differences in susceptibility. Infection was not entirely uniform, but the only differences that could be determined were that the disease was more prevalent in the lower and wetter parts of the nursery and on the shorter plants, regardless of variety. A second planting of one strain, just past emergence at the time of infection, had a much higher percentage of infected plants than the older, and taller, planting.

In the present investigation another species of *Pythium* has been shown capable of causing the same type of disease in the greenhouse as that caused by *P. ultimum* but with less severity. Probably other species could be found that would likewise be infectious under similar conditions. *P. ultimum* is the only species that has been isolated from diseased material. According to Middleton (*8*), *P. ultimum* is the commonest species of *Pythium*, and this may account in part for the fact that it was the only species isolated.

SUMMARY

A new disease of garden pea incited by *Pythium ultimum* is described. Infection occurs in the buds or in the axils of young leaves. Affected tissues collapse quickly and become straw-colored to black. Usually the upper three to five internodes are killed and new branches are put out below.

Typical disease was produced in the greenhouse by inoculating potted pea plants.

High humidity was necessary for infection.

Another species of *Pythium* was found able to cause the same type of disease but with less severity.

Temperatures from 24° to 28° C. were most favorable for disease development. The optimum for damping-off of pea seedlings and growth in culture was 28° C.

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